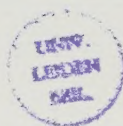


Studies on the Hepatic Metabolism of Chylomicron Phospholipids and Chylomicron Remnants in the Rat

Britta Landin



Lund 1984



STUDIES ON THE HEPATIC METABOLISM
OF CHYLOMICRON PHOSPHOLIPIDS AND CHYLOMICRON REMNANTS
IN THE RAT

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Abstract The metabolism of ^{32}P -labelled chylomicron phosphatidylcholine and phosphatidylethanolamine was studied in intact rats, in perfused livers and in isolated liver cells. After chylomicron injection into intact rats a transient increase in plasma phosphatidylethanolamine concentration occurred. The labelled phosphatidylethanolamine was eliminated from plasma more rapidly than the phosphatidylcholine and a higher fraction was recovered in the liver. The preferential uptake of phosphatidylethanolamine was correlated with the hepatic lipase activity in perfused rat livers and in isolated liver cells. The important role for hepatic lipase in the metabolism of chylomicron phosphatidylethanolamine was also demonstrated in intact rats injected with antiserum against hepatic lipase. A lysosomal retinyl ester hydrolase able to degrade chylomicron remnant retinyl esters was demonstrated in rat liver. Porta-caval shunt in the rat affected the hepatic uptake of chylomicron remnants less than would be expected from the reduction of liver mass occurring as a consequence of the operation, since the uptake of remnants per gram liver was significantly increased in the shunted animals. Liver membranes from porta-caval shunted rats showed a higher degree of calcium-dependent binding of human LDL than did membranes from normal rat livers. Bile duct ligation severely inhibited the hepatic uptake of chylomicron remnants in the rat. No inhibition of remnant uptake was seen when only one of the bile duct branches was ligated. The formation of chylomicron remnants was not affected by bile duct ligation. Intravenous infusion of bile or bile acids into normal rats also significantly decreased the hepatic uptake of chylomicron remnants.			
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Date April 2, 1984

From the Department of Physiological Chemistry,
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Forskning är från flera synpunkter
en säregen verksamhet ...

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- II. B. Landin, Å. Nilsson, J.S. Twu, M.C. Schotz
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INTRODUCTION

In plasma lipids are transported bound to protein. Free fatty acids are mainly complexed with albumin, while cholesterol, triacylglycerols and phospholipids together with certain proteins (the apolipoproteins) constitute the lipoproteins. These particles are usually divided according to their densities in four main groups: chylomicrons ($S_f > 400$, $d < 1.006$ g/ml), very low density lipoproteins (VLDL, S_f 20-400, $d < 1.006$ g/ml), low density lipoproteins (LDL, S_f 0-20, $1.006 < d < 1.063$ g/ml) and high density lipoproteins (HDL, $1.063 < d < 1.21$ g/ml), which have certain features in common, but also differ markedly in size and composition and have different roles in lipid metabolism.

Lipoproteins have been extensively studied in the rat. In many respects findings in this species may also be applied to man, but there are also several quantitative and qualitative differences between the two species. A major difference is that in rat plasma the levels of LDL, the major cholesterol-bearing lipoprotein in man, are very low, whereas HDL is the predominant lipoprotein class.

During the last years several review articles have been concerned with the structure and metabolism of the lipoproteins (Jackson et al., 1976; Scanu and Landsberger, 1980; Nilsson-Ehle et al., 1980; Green and Glickman, 1981).

Chylomicrons are large lipoprotein particles (> 1000 Å diam.) with a high fat content and thus a low density (< 0.95 g/ml). They contain mainly triacylglycerols (80-95% of particle weight) which are located in an inner core together with smaller amounts of other non-polar lipids such as cholesteryl esters (0.8-1.4%) and retinyl esters. The polar surface coat of the particle contains mainly phospholipids (6-8%) but also small amounts of unesteri-

fied cholesterol (0.8-1.6%) and protein (1-1.5%). Phosphatidylcholine (lecithin) is the major phospholipid (70-80%), but the proportion of phosphatidylethanolamine (12-16%) is significantly higher than in other plasma lipoproteins. Chylomicrons are transported via the lymph to the blood circulation where much of the chylomicron triacylglycerols is rapidly hydrolyzed by the lipoprotein lipase in the capillaries of extrahepatic tissues. The fatty acids thus liberated are either oxidized or reincorporated into tissue lipids. During the triacylglycerol hydrolysis the chylomicrons decrease in size and a loss of surface material occurs. The resulting lipoproteins, containing all of the chylomicron cholesteryl esters but only minor amounts of the original triacylglycerols and phospholipids, are called chylomicron remnants. These particles are very rapidly taken up in the liver, presumably via a lipoprotein receptor that recognizes apolipoprotein E. Most of the dietary cholesterol is thus directed into the liver where it can be incorporated into cell membranes and newly synthesized lipoproteins or excreted into the bile, either in the form of intact cholesterol or after conversion to bile acids. Chylomicron remnant cholesterol may also act as an inhibitor of the hepatic cholesterol synthesis.

VLDL (300-800 Å diam.) are smaller than chylomicrons and have a lower triacylglycerol content. Although they are mainly synthesized in the liver, VLDL is the dominant lipoprotein class produced in the intestine when dietary fat is scarce or absent. While the general structure of hepatic VLDL and intestinal VLDL and chylomicrons is the same, the apolipoprotein composition is different. The major HDL apolipoprotein, apoA-I, is the predominant apolipoprotein in the intestinal lipoproteins isolated from the lymph, whereas hepatic VLDL contain little apoA-I and a large proportion of apoE. VLDL metabolism in the rat follows the scheme outlined for chylomicrons, but the proportion of VLDL remnant particles, called IDL (intermediate density lipoproteins), that

is metabolized by the liver varies among different species. In the rat IDL are rapidly metabolized by the liver like chylomicron remnants, whereas in man a large part of the IDL remains in the circulation and is further converted into LDL.

LDL (200-250 Å diam) in plasma are mostly derived from VLDL, but some contribution may also occur by direct hepatic secretion. LDL contain mainly cholesterol and protein. Cholesterol initially derived from triacylglycerol-rich lipoproteins is transported with LDL both to the liver and other tissues. The uptake of LDL occurs partly via specific lipoprotein receptors, which are under metabolic regulation, and partly by non-specific uptake into cells belonging to the reticuloendothelial system. The apoB,E-receptor involved in LDL uptake recognizes apolipoprotein B, the only protein component in human LDL.

HDL are small particles (< 200 Å diam.) that contain mainly cholesterol, phospholipids and protein. Nascent HDL are synthesized both in the liver and in the intestine, but much of the content in the circulating HDL particles has been acquired from other lipoproteins in plasma. Two major types of HDL, called HDL₂ and HDL₃, can be isolated from plasma, the former particles having a higher lipid content and hence a lower density. HDL act as an acceptor of both surface material from triacylglycerol-rich lipoproteins and of cholesterol from extrahepatic tissues. HDL is the site of action of lecithin-cholesterol acyltransferase (LCAT), an enzyme that hydrolyses phosphatidylcholine while esterifying cholesterol. Phospholipids and cholesterol can also be delivered by HDL to the liver, where the hepatic lipase, by hydrolyzing phospholipids, contributes to the conversion of HDL₂ into HDL₃. HDL also function as a plasma reservoir for many of the apolipoproteins. It is thus evident that HDL act as a vehicle

for different lipids and apolipoproteins which can be metabolized at rates independent of the catabolism of the entire HDL particles.

This thesis is concerned mainly with two aspects of chylomicron metabolism. First, the metabolism of the predominant chylomicron phospholipids, i.e. phosphatidylcholine and phosphatidylethanolamine, was investigated. Much of these phospholipids is transferred to HDL during the formation of chylomicron remnants and thus metabolism of chylomicron phospholipids occurs partly as a consequence of the action of different enzymes on HDL. Second, the endocytotic uptake of chylomicron remnants was studied. These investigations concerned the role of lysosomal enzymes in the hydrolysis of chylomicron retinyl esters and pathophysiological aspects of remnant degradation in rats with bile duct ligation or porta-caval shunt.

BACKGROUND

A. Chylomicron synthesis

Chylomicrons are synthesized in the enterocytes during fat absorption and transport both dietary lipids and lipids derived from the bile and de novo synthesis in the mucosal cells via the lymph to the blood circulation.

Hydrolysis of triacylglycerols in the intestinal lumen yields fatty acids and 2-monoacylglycerols which are absorbed into the enterocytes. Triacylglycerols are then synthesized in the smooth endoplasmic reticulum. Part of the cholesterol incorporated in the chylomicrons is esterified in the enterocytes. The esterifying activity has been attributed both to the pancreatic cholesterol esterase (Gallo et al., 1977) and to the action of an acyl-CoA: cholesterol acyltransferase (ACAT) in the mucosal cells (Haugen and Norum, 1976).

Phosphatidylcholine is also hydrolyzed in the intestinal lumen, and the resulting 1-acylglycerophosphorylcholine and fatty acid are absorbed. Most of the chylomicron phosphatidylcholine is formed by reacylation of the lysophosphatidylcholine, although when the supply of luminal phospholipids is limited a significant de novo synthesis occurs (Mansbach, 1977). An uptake of intact phosphatidylcholine molecules from the bile has been suggested (Boucrot, 1972), although such a process - if existing - seems to be of no quantitative significance (Larsson and Nilsson, 1978). The fatty acid composition in chylomicron phospholipids is, in contrast to the triacylglycerols, little affected by dietary variations (Whyte et al., 1963; Arvidson and Nilsson, 1972). The chylomicron phospholipid composition is 69-78% phosphatidylcholine, 12-16% phosphatidylethanolamine, 2-12% sphingomyelin and 3% lysophosphatidylcholine (Zilversmit, 1965; Kostner and Holasek, 1972; Klein and Rudel, 1983).

Fat-soluble vitamins are partly transported with the chylomicrons, although also transport via the vena porta occurs. Retinol is transported in esterified form with the chylomicrons (Goodman et al., 1965). Also vitamin D₃ and 25-hydroxy-vitamin D₃ are transported with the chylomicrons into the lymph, although most of these compounds are transported in the unesterified form (Maislos et al., 1981) and are rapidly transferred to binding proteins (Dueland et al., 1983).

Patients with abetalipoproteinemia are unable to secrete chylomicrons, and apoB can not be detected in their intestinal mucosa (Schwartz et al., 1978; Glickman et al., 1979). In the rat also, chylomicron secretion seems to depend on intact intestinal apolipoprotein synthesis (Glickman et al., 1972), although this study does not determine the relative importance of various apolipoproteins. The intestine synthesizes considerable amounts

not only of apoB, but also of apoA-I (Rooke and Skinner, 1976; Glickman and Green, 1977). Fat absorption increases the mucosal content of apoA-I (Glickman and Green, 1977), but apoA-I synthesis seems to occur also in the absence of active fat absorption (Bearn et al., 1982; Windmueller and Wu, 1981). The rat intestine also produces apoA-IV (Holt et al., 1979). In contrast to the findings in man (Rachmilewitz et al., 1978) a significant synthesis of apoA-II does not occur in the rat intestine (Wu and Windmueller, 1978). Most of the apoC found in lymph chylomicrons is probably synthesized in the liver, but a substantial proportion of the apoC-II content seems to arise from the intestine (Holt et al., 1979; Fidge and Kimpton, 1983).

The protein composition of rat chylomicrons isolated from mesenteric lymph has been determined after gel chromatography. The apoB then constitute 8%, apoA-IV 8%, apoC 38%, and apoE and apoA-I together 38% of the original protein. Using radioimmunoassay 4% of the total protein was apoE and 31% apoA-I (Imaizumi et al., 1978). This distribution largely resembles that reported by Holt et al. (1979) and Fidge and McCullagh (1981), although the latter study also demonstrated a significant contribution of a new apolipoprotein designated apoA-V. While apoB in plasma is heterogeneous (Elovson et al., 1981), only one form of relatively low molecular weight is found in rat chylomicrons (Krishnaiah et al., 1980), probably corresponding to the apoB-48 described in human chylomicrons (Kane et al., 1980).

B. Intravascular metabolism of chylomicrons

Lipoprotein lipase is located on the endothelial walls in the capillaries of most extrahepatic tissues. This enzyme which is under hormonal control, hydrolyzes the chylomicron triacylglycerols to yield fatty acids and 2-monoacylglycerol. Most of the fatty acids are transported through the endothelial cells into the tissue, where they are utilized for oxidation or incorpora-

tion into storage lipids. The resulting monoacylglycerols could be hydrolyzed by lipoprotein lipase only after isomerization. Since this probably is a slow process and no accumulation of monoacylglycerols occurs, distinct monoacylglycerol hydrolases might be of importance (Tornqvist et al., 1978; Bry et al., 1979). ApoC-II is a necessary cofactor for the lipoprotein lipase activity and the total lack of apoC-II leads to severe hypertriglyceridemia in man (Breckenridge et al., 1978). The apoC-II content is not normally rate-limiting for the hydrolysis of chylomicron triacylglycerols (Fielding and Fielding, 1976) although the activity of lipoprotein lipase is progressively decreased against the particles as they become depleted of triacylglycerols during the course of hydrolysis (Higgins and Fielding, 1975).

As a consequence of the exposure to plasma and the action of lipoprotein lipase the native chylomicrons are transformed into another type of lipoprotein - the chylomicron remnants. This term was originally coined by Redgrave (1970), who extended the earlier investigations in hepatectomized dogs performed by Nestel et al (1963). Redgrave (1970) thus demonstrated that in the rat most of the chylomicron triacylglycerols are metabolized extra-hepatically, leaving a remnant particle enriched in cholesteryl esters, which is more readily taken up in the liver than chylomicrons. Mjös et al. (1975) further characterized the remnants formed during hydrolysis of large chylomicrons in vivo. The lipoprotein diameter was then reduced to 200-1200 from the initial 700-3000 Å, though most of the remnants were still recovered in the $d < 1.006$ g/ml fraction. The composition of these chylomicron remnants was, on a weight basis, 70% triacylglycerols, 10% phospholipids, 5% cholesterol, 6% cholesteryl esters and 9% protein. During remnant formation the relative content of apoE increased while the apoC decreased, in comparison with the native chylomicrons which had been incubated with HDL in vitro.

Later it was demonstrated by Florén et al. (1981) that human chylomicrons isolated from plasma from a patient with familial hypertriglyceridemia lose apoE during the triacylglycerol hydrolysis in vitro. Since a more extensive loss of apoC occurs concomitantly, the ratio of apoE to apoC increases.

C. Metabolism of polar surface components

During in vitro incubation of chylomicrons with serum the total chylomicron protein content increases because of a gain in apoE and apoC (Imaizumi et al., 1978). The relative content of apoA-I is thus decreased in the chylomicrons (Robinson and Quarfordt, 1978; Imaizumi et al., 1978; Nilsson et al., 1981) although no net loss of apoA-I occurs (Imaizumi et al., 1978; Vigne and Havel, 1981). The chylomicron phospholipid content was lowered during the incubation (Imaizumi et al., 1978), thus confirming the previously observed transfer of chylomicron phospholipids to HDL in vitro (Minari and Zilversmit, 1963).

Havel (1957) observed a substantial postprandial increase in HDL phospholipids in man after a fat meal. An increased phospholipid content in the ingested meal does not influence the magnitude of this HDL phospholipid increment (Simonsson et al., 1982). It has been demonstrated in the rat that phospholipids (Mjös et al., 1975; Redgrave and Small, 1979), apoA-I and possibly also apoA-IV (Tall et al., 1979, Vigne and Havel, 1981) are transferred from the chylomicrons during remnant formation. These surface components form vesicles which can transiently be isolated in the LDL range (Tall et al., 1979; Tall et al., 1980), although the material is later incorporated into preexisting HDL (Redgrave and Small, 1979; Tall et al., 1980). A significant increase in HDL phospholipid mass is not seen until 10 min after chylomicron injection in the rat (Tall et al., 1979).

The apoA-I derived from the chylomicrons can thus be expected to be metabolized as part of the HDL. The half-life of iodinated rat HDL in plasma is about 10 h (Roheim et al., 1972; Sigurdson et al., 1979) and there are no major discrepancies between the elimination rates of different HDL apolipoproteins (Eisenberg et al., 1973). Chylomicrons in plasma gain both apoE and apoC but during the triacylglycerol hydrolysis much of these apolipoproteins is transferred back to the HDL reservoir, from which they can probably again be recruited to newly entering triacylglycerol-rich lipoproteins.

Scow and Egelrud (1976) have demonstrated that about 75% of the phosphatidylcholine content in native chylomicrons can be hydrolyzed by lipoprotein lipase in vitro. The significant part of the chylomicron phospholipids that is transferred to HDL in vivo is, however, likely to escape hydrolysis by lipoprotein lipase, since HDL phospholipids are not hydrolyzed by this enzyme in vitro (Eisenberg et al., 1978). In the hepatectomized rat, 30 min after chylomicron injection there is no gross accumulation of lysophosphatidylcholine, and >90% of the injected chylomicron phosphatidylcholine radioactivity is retained in plasma (Redgrave and Small, 1979). In the intact animal, however, at this time about 30% of the labelled phosphatidylcholine is recovered in the liver (Redgrave and Small, 1979).

An important role in the degradation of HDL phospholipids has been ascribed to the hepatic lipase (Kuusi et al., 1979a; Jansen et al., 1980b) located on the endothelial cells in the liver (Kuusi et al., 1979b; Jansen et al., 1980a). This enzyme readily hydrolyzes HDL phospholipids in vitro (Van Tol et al., 1980; Shirai et al., 1981). Several investigators have demonstrated an increased level of HDL phospholipids after administration of antiserum against hepatic lipase in the rat (Kuusi et al., 1979a; Jansen et al., 1980b; Grosser et al., 1981). The increase is

confined to the HDL₂ region (Jansen et al., 1980a) and this lipoprotein is also the favoured substrate for hepatic lipase in vitro (Groot et al., 1981a; Shirai et al., 1981).

D. Hepatic metabolism of chylomicron remnants

Chylomicron remnants are metabolized by receptor-mediated endocytosis and subsequent lysosomal degradation in the liver (for refs. see Florén, 1978; Brown et al., 1981). Although the nature of the hepatic receptor involved remains to be clarified, there is considerable evidence indicating that the apoE of the chylomicron is of importance as a ligand for receptor-binding. In the rat, the binding to liver membranes of lipoproteins containing only apoB, such as human LDL, is very low (Kovanen et al., 1979). Treatment with high doses of 17 α -ethinylestradiol markedly increases the clearance of lipoproteins containing apoB and/or apoE (Chao et al., 1979) by increasing the capacity for binding of such lipoproteins to liver membranes (Kovanen et al., 1979; Windler et al., 1980b). The apoB,E-receptor induced by estrogen treatment is located in the hepatocytes (Chao et al., 1981; Harkes and Van Berkel, 1983) and has been shown to resemble the LDL-receptor (Kovanen et al., 1981; Beisiegel et al. 1981) previously described by Goldstein and Brown (1977) in fibroblasts. Another lipoprotein receptor which recognizes apoE but not apoB has been described in livers of dog, swine and man (Hui et al., 1981; Mahley et al., 1981). This apoE-receptor is probably also present in the rabbit (Kita et al., 1982) and in the rat (Cooper et al., 1982a) liver, where it is responsible for the rapid uptake of chylomicron remnants. This binding seems to depend entirely on the presence of apoE in the remnant particles (Sherrill et al., 1980) and is not affected by a lack of apoB (Borensztajn et al., 1982).

Even if apoE is the sole ligand involved in the binding of chylomicron remnants to liver cells, this binding can probably be modulated by other apolipoproteins. First, different isoforms of apoE have been shown to differ in their ability to bind to the apoB,E-receptor (Havel et al., 1980). Second, an increased content of apoC-III inhibits the effect of apoE in triacylglycerol-rich lipoproteins (Shelburne et al., 1980; Windler et al., 1980a; Van Berkel et al., 1983). Thus the ratio of apoE to apoC, rather than the absolute amount of apoE in a particle, determines the efficiency with which it will be taken up in the liver. Third, it has been suggested that the apoB variant could be of importance for determining the rate of hepatic uptake (Elovson et al., 1981; Sparks and Marsh, 1981; Wu and Windmueller, 1981). However, comparison of the metabolism of chylomicrons from intestinal lymph and VLDL isolated from liver perfusates reveals no correlation between the rate of removal and the origin of the lipoproteins and thus to their apoB composition (Cooper et al., 1982b). Fourth, treatment of chylomicrons with phospholipase which leads to a loss of most of their phospholipid content without changing the apolipoprotein composition, significantly increases the uptake of chylomicrons in rat livers (Borensztajn et al., 1980; Borensztajn and Kotlar, 1981). Although the mechanism for this increased binding is not clear, it has been proposed that the removal of phospholipids from the lipoprotein surface might render the apoE more accessible for binding to hepatic receptors.

Hepatic lipoprotein uptake can also be regulated by the expression of lipoprotein receptors. As mentioned earlier, the apoB,E-receptor activity is normally very low in rat liver, although it can be significantly increased by treatment with large doses of ethinylestradiol. In the growing dog, the expression of this receptor is inversely correlated to the age of the animal (Mahley et al., 1981) and in adult dogs the apoB,E-recep-

tor can be induced by prolonged fasting or treatment with cholestyramine, colestipol or mevinolin (Mahley et al., 1981; Hui et al., 1981; Kovanen et al., 1981). Such hypocholesterolemic drugs also stimulate the receptor-dependent elimination of LDL in man and rabbit (Slater et al., 1980; Chao et al., 1982; Bilheimer et al., 1983). On the other hand cholesterol-feeding decreases the expression of the apoB,E-receptor in immature dogs (Mahley et al., 1981). Apart from these long-term effects, the induced apoB,E-receptor can be very rapidly down-regulated by infusion of lymph or bile acids in the dog (Angelin et al., 1983). In contrast, these studies have not revealed any sign of metabolic regulation of the apoE-receptor (Mahley et al. 1981; Angelin et al., 1983). Also, the clearance of chylomicrons from plasma and the uptake of chylomicron remnants in the liver is not significantly affected by cholesterol-feeding in the dog (Melchior et al., 1981).

Besides the hepatocytes which comprise 78% of the total rat liver volume, this organ also contains non-parenchymal cells, i.e. Kupffer cells, endothelial cells and fat-storing cells. These latter cell types occupy 6.3% of the liver volume, while the remaining 15.7% is extracellular space (Blouin et al., 1977). Early autoradiographic studies using cholesterol-labelled chylomicrons indicate that the cell type mainly responsible for the uptake of chylomicron remnants in the liver is the hepatocyte (Stein et al., 1969). Following injection of cholesterol-labelled lymph only about one tenth of the total liver radioactivity is found in the Kupffer cells (Redgrave, 1970; Nilsson and Zilver-smit, 1971; Lippiello et al., 1981). In recent studies using chylomicron remnants labelled in the apoB moiety, the non-parenchymal liver cells have been estimated to contribute about 35% of the total hepatic uptake (Groot et al., 1981b).

According to the principles outlined for the receptor-dependent LDL metabolism the lipoproteins are catabolized in the lysosomes after receptor-mediated endocytosis (Brown et al., 1975). The degradation of chylomicron cholesteryl esters (Florén and Nilsson, 1977) and protein (Florén and Nilsson, 1978) in rat hepatocytes is inhibited by administration of chloroquine, a drug that inhibits lysosomal degradation (de Duve et al., 1974). Rat liver lysosomes efficiently hydrolyze both chylomicron remnant triacylglycerols and cholesteryl esters in vitro (Nilsson, 1976). A lysosomal lipase active against these two substrates has been partly purified from rat (Teng and Kaplan, 1974) and human liver (Warner et al., 1981).

E. Chylomicron metabolism in pathological states of the liver

Several studies have dealt with the changes in plasma lipids and lipoproteins in liver disease (for refs. see McIntyre, 1978), although very little attention has been paid to the metabolism of chylomicrons in these states. Obstructive jaundice is generally associated with hypercholesterolemia and an increased hepatic cholesterol synthesis. Studying rat plasma after two days of biliary obstruction, Calandra and coworkers (1975) have demonstrated increased levels of both VLDL, LDL and HDL. All lipoprotein classes contain increased amounts of phospholipids and unesterified cholesterol, although in the VLDL fraction there is also a significant increase in cholesteryl esters. Several pathological lipoproteins have been isolated in the LDL region of cholestatic plasma. The most intensively studied is the lipoprotein X (LP-X) (Seidel et al., 1969), which is found not only in man but also secreted from the cholestatic rat liver (Felker et al., 1978). This particle contains equal proportions of phospholipids and unesterified cholesterol, together constituting about 90% of the particle (Felker et al., 1978). A triacylglycerol-rich particle, designated β_2 -LP (Müller et al., 1974) or LP-Y (Kostner et al., 1976) has been demonstrated in plasma from patients with

cholestatic jaundice. Since this particle disappears when fat intake is limited it has been suggested that it arises from chylomicron metabolism (Müller et al., 1974). However, a lipoprotein resembling the LP-Y is secreted from the perfused cholestatic rat liver (Felker et al., 1978). Bile acids bind to HDL in cholestatic plasma and it has been proposed that this changes the distribution of apoA-I from these lipoproteins to the $d=1.21$ g/ml infranatant (Middelhoff et al., 1979). Disc-shaped HDL also appear in cholestasis (Pasquali-Ronchetti et al., 1975) and thus the lipoprotein changes seen in this condition in many ways resemble the changes seen in LCAT deficiency (Norum et al., 1972). In spite of this, the LCAT activity is mostly normal or increased in cholestatic plasma (Calandra et al., 1972; Kepkay et al., 1973).

Treatment with D-(+)galactosamine induces a hepatocellular injury in the rat (Keppler et al., 1968), and thereby the production of LCAT is decreased. In fact most of the lipoprotein changes seen after such treatment (Cartwright et al., 1982) can be explained by a reduced LCAT activity. In the galactosamine-treated animals there is, however, also an accumulation of chylomicron triacylglycerols in plasma (Black et al., 1982), although the uptake of preformed chylomicron remnants during liver perfusion is little affected (Manowitz et al., 1983). In this condition defective chylomicron clearance may be caused by a reduced activity of lipoprotein lipase, though impaired activity of hepatic lipase (Black et al., 1982) and the reduced levels of apoC and apoE (Black et al., 1983) might also be of importance.

The establishment of a porta-caval anastomosis is known to induce hypolipidemia and particularly to lower an increased plasma cholesterol level in several species (for refs. see Beher and Toledo-Pereyra, 1980). The beneficial effect of porta-caval shunt in patients with hyperlipoproteinemia has been attributed

to a decreased hepatic cholesterol synthesis, although studies on this point in different species have given conflicting results (Beher and Toledo-Pereyra, 1980). Several investigators have demonstrated that the main reduction of cholesterol or protein content following porta-caval shunt in the rat is confined to the $d < 1.006$ g/ml fraction (Magide et al., 1976; Bützow et al., 1977; Magot et al., 1983). Studies on the metabolism of triacylglycerol-rich lipoproteins in this condition, however, have hitherto not been performed.

PRESENT INVESTIGATIONS

A. Metabolism of chylomicron phosphatidylcholine and phosphatidylethanolamine (I,II)

The triacylglycerol-rich lipoproteins secreted by the intestine transport considerable amounts of phospholipids. The phospholipid to apoA-I weight ratio in lymph chylomicrons is about 30 (Imaizumi et al., 1978), which is far higher than in both plasma HDL and nascent HDL isolated from rat liver or intestine, where the phospholipid to protein ratio never exceeds 2 (Hamilton et al., 1976; Green et al., 1978). It is thus evident that although much of the circulating HDL phospholipids is derived from the chylomicrons, a quantitatively important catabolism of phospholipids must occur without a simultaneous degradation of apoA-I. Accordingly, the turnover rate of chylomicron phospholipids must be significantly higher than that of apoA-I in HDL. It has been estimated by Redgrave and Small (1979) that injection of chylomicrons in a dose corresponding to the amount produced during 1h, into a hepatectomized rat, leads to an increase in the HDL phospholipid pool by about 25%. This is a greater increase than that actually seen in intact rats, where the liver may remove larger chylomicron remnants with a greater content of surface material than those accumulating in plasma of the hepatectomized

animal. Furthermore, the liver may also provide pathways for clearance of a greater amount of phospholipids than that amount which is transported with the chylomicron remnants.

Earlier studies on the metabolism of chylomicron phospholipids have focused on the fate of total phospholipids (Tall et al., 1979) or phosphatidylcholine (PC) (Redgrave and Small, 1979). These studies have not considered other phospholipids such as phosphatidylethanolamine (PE) which is more abundant in chylomicrons than in other lipoproteins. For instance, in rat chylomicrons the ratio of PE to PC is 0.16 ± 0.02 ($n=5$), while in normal rat HDL₂ the ratio is less than 0.03 (Van Tol et al., 1980).

After injection of ^{32}P -labelled chylomicrons into intact rats a greater fraction of the injected [^{32}P]PE than of the [^{32}P]PC disappeared from plasma at all times studied (I), although in absolute terms the rate of removal of [^{32}P]PC is about three-fold greater than that of [^{32}P]PE. A significant clearance of radio-labelled chylomicron phospholipids from plasma occurs within a few minutes after injection. For instance, 20% of the [^{32}P]PC and 40% of the [^{32}P]PE is removed during the first minute, while 30 min later 40% of the labelled PC but only about 5% of the PE is still retained in plasma (I).

The relative contribution of net removal and phospholipid exchange, to the elimination of radiolabelled phospholipids from plasma, can not be easily determined since the endogenous pool of total plasma phospholipids or PC is about tenfold higher than the dose injected. However, the higher ratio of PE to PC in chylomicrons than in other lipoproteins, allows the mass elimination of chylomicron PE to be followed. It can thus be demonstrated that about 50% of the injected chylomicron PE is cleared from plasma in 5 min, and already 10 min after the injection the plasma PE

concentration has returned to normal (I). These findings indicate that the rapid clearance of [^{32}P] PE is primarily due to metabolism and not to phospholipid exchange.

It is a subject of some controversy, whether there is also a net transfer of phospholipids to HDL in the absence of lipolysis (Minari and Zilversmit, 1963; Redgrave and Small, 1979). In the present studies HDL were prepared by incubating ^{32}P -labelled chylomicrons with serum. The PE to PC ratio in HDL after the incubation was about 50% of the corresponding ratio in the added chylomicrons, both when expressed as mass and as radioactivity, thus indicating that some net transfer, at least of PE, occurs during the incubation.

Five minutes after the injection of radiolabelled chylomicrons into intact rats, more than 80% of the labelled PC and an equal fraction of PE is eliminated from the chylomicrons (I). Since similar proportions of PC and PE mass are removed from the chylomicrons (I), this suggests that both phospholipids are released from the chylomicrons as integral parts of surface fragments. Although equal proportions of PC and PE are removed from the chylomicrons, a lesser fraction of the [^{32}P] PE than of the [^{32}P] PC appears in denser lipoproteins. Therefore a higher proportion of the PE than of the PC must be removed from plasma either before, or very early after, its incorporation into HDL.

Lipoprotein lipase can readily degrade chylomicron PC in vitro (Scow and Egelrud, 1976), and with artificial substrates its activity against PE is 10 times higher than against PC (Groot et al., 1978). Also during VLDL hydrolysis in vitro the degradation of PE seems to be preferred over PC, especially when the triacylglycerol hydrolysis is not extensive (Eisenberg and Olivecrona, 1979). Prior to incorporation into HDL, chylomicron surface constituents can be isolated in vesicular form from the LDL

region (Tall et al., 1979). Such material can possibly also be a substrate for lipoprotein lipase. During hydrolysis of VLDL with lipoprotein lipase in the absence of serum, similar surface fragments accumulate and although their phospholipids can be degraded by lipoprotein lipase they seem to be less efficiently hydrolyzed than when present in the original VLDL (Eisenberg and Olivecrona, 1979).

Table I

Metabolism of chyle PC and PE in the hepatectomized rat.

	Relative recovery in plasma (%)	
	-heparin (n=10)	+heparin (n=4)
Triacylglycerols	51 $\pm$ 11	7 $\pm$ 1
PC	120 $\pm$ 11	129 $\pm$ 8
PE	93 $\pm$ 9	85 $\pm$ 11

Functionally hepatectomized rats were injected with lymph labelled with [^{32}P]phosphate and [^{14}C]linoleic acid. 10 min after the injection the recoveries of [^{14}C]triacylglycerols, [^{32}P]PC and [^{32}P]PE in plasma were compared to the recovery of simultaneously injected [^3H]retinyl or [^3H]cholesteryl ester labelled chyle. The recovery of these latter compounds was assigned the value 100 %. In some experiments the animals were injected with 250 U heparin after the hepatectomy but immediately before the chyle injection.

Lipoprotein lipase activity could thus well contribute to the removal of both PC and PE very early after chylomicron injection, although once the phospholipids are transferred to HDL they are probably no longer susceptible to the action of lipoprotein lipase (Eisenberg et al., 1978). Injection of heparin greatly promotes the elimination of chylomicron triacylglycerols in the

hepatectomized rat, without significantly affecting the removal of either PC or PE (Table I). Thus the degradation of chylomicron phospholipids by lipoprotein lipase is not extensive under these conditions.

Table II

Distribution of ^{32}P phospholipids in plasma 10 min after chyle injection.

	PC	PE	Lyso-PC
Chyle, injected (16)	63 ± 1	25 ± 1	1.6 ± 0.1
Plasma, normal rats (6)	66 ± 4	12 ± 2	7 ± 0.5
Plasma, hepatectomized rats (10)	64 ± 2	18 ± 2	8 ± 1
Plasma, hepatectomized rats + heparin (4)	66 ± 4	14 ± 1	13 ± 1

The rather small lyso [^{32}P]PC accumulation in plasma of the hepatectomized animals (Table II; Redgrave and Small, 1979) supports this conclusion, and also indicates that no extensive metabolism of chylomicron PC by LCAT occurs. Since the activity of LCAT against PE is very low in vitro (Nichols and Gong, 1971; Fielding, 1974), this enzyme is not likely to catalyze the rapid clearance of [^{32}P]PE which occurs also after its transfer to HDL (I). Rather the in vivo experiments point to a role for the liver and for a pathway that preferentially degrades PE, i.e. they suggest a role for the hepatic lipase.

Experiments on perfused rat livers or isolated liver cells indicate that the preferential PE uptake correlates with the hepatic lipase activity (I). Injection of antiserum against

hepatic lipase into intact rats has further confirmed the role for this enzyme in the clearance of PE (II). Inhibition of the hepatic lipase thus retards the elimination of radiolabelled PE from chylomicrons and even more so from HDL. However, after antiserum treatment a rapid initial removal of radiolabelled chylomicron PE from plasma also occurs, thus demonstrating the contribution of lipoprotein lipase to the PE elimination.

It is well established that antiserum against hepatic lipase increases the HDL₂ phospholipid content (Kuusi et al., 1979a; Jansen et al., 1980b; Grosser et al., 1981), but the distribution of different phospholipids has not been determined. In women, estrogen treatment causes an elevation of both plasma PC and PE, and it has been suggested that this depends on a reduced hepatic lipase activity (Silfverstolpe et al., 1981). Although the considerable increase in plasma PE seen after antiserum treatment (II) might significantly contribute to the increase in total phospholipids, an increased level of the most abundant phospholipid, i.e. PC, is also expected. Indeed, an increased plasma concentration of PC was seen in animals injected with HDL and antiserum, although the disappearance of radiolabelled PC was not significantly affected (II). Even if a smaller fraction of PC than of PE is degraded by the action of hepatic lipase, the greater mass of PC transported in plasma would, over long time periods, lead to a considerable PC accumulation when the hepatic lipase is inhibited.

Taken together the data indicate that very early after chylomicron injection, when both PC and PE are still present at the chylomicron surface, the two phospholipids can be hydrolyzed by lipoprotein lipase, which probably degrades a somewhat higher fraction of PE than of PC. In a few minutes, the major part of both phospholipids is detached from the chylomicrons as part of surface fragments which can possibly also be a substrate for

lipoprotein lipase, although once these fragments fuse with preexisting HDL, the lipoprotein lipase is no longer able to attack the phospholipids. Instead the hepatic lipase removes almost all of the remaining PE and also a substantial fraction of PC, although part of this phospholipid can also be hydrolyzed by LCAT in HDL. The minor contribution of the LCAT pathway to the PC removal is demonstrated by the significant rise in plasma PC after injection of antiserum against hepatic lipase. Also, if all PC transported with the intestinal lymph during 1 h in a fasting rat (about 1.5 mg) was degraded by LCAT, the cholesteryl esters concomitantly formed would correspond to about one-third of the total HDL cholesteryl ester pool.

The high turnover rate of PE may reflect a more important function in terms of delivery of fatty acids to tissues than might be expected from its low plasma concentration. An interesting finding, although hard to interpret, is that PE feeding in the rat induces plasma lipoprotein changes such as decreased levels of phospholipids, cholesterol, apoA-I, and apoE, while apoB is significantly increased (Imaizumi et al., 1983). Similar changes can be induced by addition of free ethanolamine to the diet (Murata et al., 1982).

The extensive flow not only of PE but of total phospholipid mass from the intestine to the liver deserves to be considered. In spite of the lack of significant recycling of intact PC molecules in the bile (Larsson and Nilsson, 1978), and although the contribution of ^{32}P -labelled chylomicron PC to the bile is negligible (Table III), the preferential utilization of bile PC for chylomicron production (Mansbach, 1977) combined with the channelling of phospholipids from the intestine to the liver makes it justifiable to speak of an enterohepatic circulation of phospholipids. This applies, however, only to the flow of phospholipid mass and not to the behaviour of discrete phospholipid

molecules. The enterohepatic route may also transport significant amounts of metabolically important fatty acids to the liver. For instance, 16% of the fatty acids in chylomicron PC is arachidonic acid (Patton et al., 1984) and this fatty acid is also abundant in chylomicron PE (Zilversmit, 1965).

Table III

Appearance in bile of lymph [^{32}P]PC

Time (n)	Recovery per ml bile (% of injected)
0- 1	0.09 ± 0.02
1- 2	0.40 ± 0.06
2- 4	0.56 ± 0.04
4- 8	0.52 ± 0.02
8-12	0.53 ± 0.01

1 ml of ^{32}P -labelled lymph was injected intravenously into bile fistula rats. Values are means $\pm$ SEM for 3 animals.

B. Metabolism of chylomicron remnant retinyl esters (III)

Vitamin A is transported as retinyl esters with the chylomicrons from the intestine to the liver (Goodman et al., 1965), which secretes only unesterified retinol bound to retinol-binding protein (Muto and Goodman, 1972). Hence retinyl esters have been utilized as a tracer for chylomicron remnants (Ross and Zilversmit, 1977), and this would be especially appropriate in the rat where protein-mediated retinyl ester exchange in plasma (Zilversmit et al., 1982) is probably insignificant (Barter and Lally, 1978).

Evidently hydrolysis of chylomicron retinyl esters must occur in the liver. Since both the chylomicron remnant cholesteryl ester (Florén and Nilsson, 1977) and protein (Florén and Nilsson, 1978) portions are degraded by lysosomal enzymes after the receptor-mediated endocytosis of the entire remnant particle, it is conceivable that there is a lysosomal retinyl ester hydrolase.

Several investigators have reported retinyl ester hydrolyzing activities in different subcellular fractions of the rat liver (Mahadevan et al., 1966; Chen and Heller, 1979; Prystowsky et al., 1981). In none of these studies was the hydrolyzing activity examined in the acid pH range. Furthermore, only retinyl esters in unphysiological preparations have been used in the assays.

Paper III describes the hydrolysis of retinyl esters by rat liver homogenates after the administration of [^3H]retinol-labelled lymph in vivo. After 2 hrs of incubation a significant hydrolysis occurs at pH 4.5. The retinyl ester hydrolase activity is enriched in purified lysosomal fractions. The study utilizes a method for isolating lysosomes in a metrizamide gradient (Wattiaux et al., 1978) with which a very high degree of enrichment of lysosomal marker enzymes can be achieved.

The acid retinyl ester hydrolase demonstrated may be identical to the formerly described acid cholesteryl ester hydrolase of rat liver (Nilsson, 1976) and it is possible that one single enzyme degrades both the triacylglycerols, the cholesteryl esters and the retinyl esters which are transported to the liver by the chylomicron remnants. Retinol is stored in the liver mainly in the esterified form (Goodman et al., 1965). Although retinyl palmitate is the predominant ester in both chylomicrons and in the liver, there are indications that a hydrolysis and subsequent reesterification occurs in the liver (Lawrence et al., 1966). The retinyl ester hydrolases with optimal activity around pH 8 might

thus be involved in the degradation of storage retinyl esters. Retinyl ester hydrolyzing activity together with significant amounts of retinyl esters has been isolated from rat liver cytosol (Sklan et al., 1982). In this context it is of interest that a transfer of chylomicron [^3H]retinol from the hepatocytes to the stellate cells (fat-storing cells) has been described (Blomhoff et al., 1984). Whether this transfer involves free or esterified retinol is, however, not known. None of the different retinyl ester hydrolases described in the rat liver has been assigned to a specified type of liver cells.

C. Metabolism of chylomicron remnants after porta-caval shunt (IV)

Porta-caval shunting leads to a decrease in liver mass of about 50%, while the total blood flow per g liver remains unchanged (Ossenberg et al., 1974). Such a marked reduction of liver mass would be expected to interfere with the clearance of chylomicron remnants (Koelz et al., 1982). The liver uptake of chylomicron remnant cholesteryl esters is actually impaired following injection of a large load of lymph into a porta-caval shunted rat (IV). However, the hepatic uptake of moderate amounts of cholesterol, injected in the form of lymph or as preformed chylomicron remnants is not significantly affected. An efficient uptake of chylomicron remnant cholesterol can be achieved in spite of the severely reduced liver mass, since the uptake of cholesterol per g liver is markedly elevated in the porta-caval shunted rats. Also the calcium-dependent binding of human ^{125}I -LDL is higher to liver membranes prepared from porta-caval shunted rats than from sham operated animals, thus indicating an increased apoB,E-receptor activity in the porta-caval shunted animals (Fig. 1).

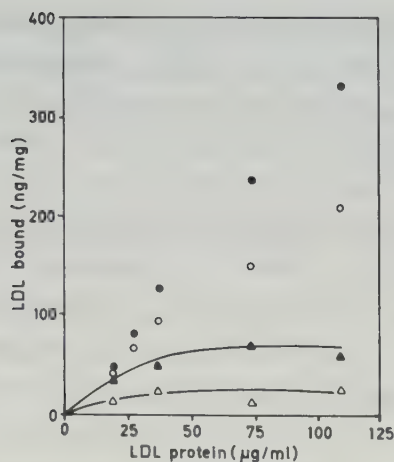


Fig. 1. In vitro binding of ^{125}I -labelled human LDL to liver membranes from porta-caval shunted rats

Incubations were performed for 1 h at 0° in a total volume of 100 μl 150 mM NaCl, 1 mM CaCl_2 , 20 mM Tris-HCl, 2% bovine serum albumin, pH 7.4, in the absence or presence of 30 mM Na_2EDTA . The ^{125}I -labelled LDL contained 35 cpm/ng protein. Membranes were prepared according to Kovanen et al. (1979) from two pooled livers from either porta-caval shunted (457 μg protein; ●, ▲) or sham operated rats (472 μg protein; ○, △). Values are means of duplicate incubations. Circles (○, ●) represent the total binding, i.e. the binding in absence of Na_2EDTA , while triangles (△, ▲) indicate the calcium-dependent specific binding, i.e. the difference between binding in absence and presence of Na_2EDTA .

Induction of the apoB,E-receptor in rat liver has only been demonstrated to occur as a result of estrogen treatment. Although the porta-caval shunted rats do have an increased estradiol concentration in plasma (IV), it is uncertain whether this influences the lipoprotein elimination since massive doses of 17α -ethinylestradiol are needed to reduce significantly the plasma cholesterol in the rat (Davis and Roheim, 1978; Wilcox et al., 1981).

In the dog, prolonged fasting is known to induce the hepatic apoB,E-receptor (Mahley et al., 1981). The animals in this study (IV) were fed ad libitum. It has previously been demonstrated that porta-caval shunted rats treated in a similar way have a considerably lower food intake than control animals even at times when the initial weight loss has ceased and the weight becomes stable (Holmin et al., 1980). The lipoprotein receptor function of the porta-caval shunted rat could thus be influenced both by the consequences of the operation and by the low caloric intake, but whether the apoB,E-receptor can be affected by dietary regulation in the rat is not known. Livers from starved rats show a higher uptake of apoE-enriched triacylglycerol emulsion particles than livers from fed rats when perfused with a serum-free medium (Quarfordt et al., 1981). This is, however, not likely to be a consequence of receptor induction, but rather depends on a greater production of apoC from the fed rat livers which may counteract the apoE-mediated hepatic uptake. Whether this finding has implications in the intact animal is uncertain. No data on the effect of porta-caval shunt on the plasma apolipoprotein composition are available.

Even in porta-caval shunted rats maintained on a lard-sucrose diet, which have a normal food intake and a normal weight, a very significant reduction of cholesterol in $d < 1.006$ g/ml lipoproteins is found (Magot et al., 1983). The cholesterol metabolism in these animals has been shown to resemble that produced by glucagon administration, and it is known that porta-caval shunted rats have a decreased insulin to glucagon ratio in plasma both when compared to pair-fed and ad libitum fed controls (Rossouw et al., 1978). Hypothetically, the hormonal effects of starvation rather than the decreased caloric intake per se, might influence the apoB,E-receptor expression. Yet, the reason for the increased uptake of chylomicron remnants per unit liver weight in the porta-caval shunted rat remains to be established. Although an

increased apoB,E-receptor activity might play a role in the rat, it should be noted that the fractional catabolic rate of homologous LDL is not affected by porta-caval shunt in swine (Carew et al., 1976).

D. Metabolism of chylomicron remnants after bile duct ligation (V,VI)

The hepatic elimination of chylomicron remnants is severely inhibited in the bile duct ligated rat (V,VI), while the clearance from plasma of chylomicron triacylglycerols is only marginally affected. Other pathological conditions that have earlier been associated with reduced chylomicron remnant clearance in the rat are streptozotocin-induced diabetes (Redgrave and Snibson, 1977) and hypercholesterolemia induced in hypothyroid rats (Redgrave and Snibson, 1977; Florén and Nilsson, 1981). The severity of the inhibition is, however, more pronounced in cholestasis than in the other conditions.

Several factors must be considered when trying to discover the mechanism responsible for the inhibition of remnant uptake in cholestasis. Obviously the properties of the chylomicron remnants, such as the apoE to apoC ratio (Windler et al., 1980a; Shelburne et al., 1980) and the phospholipid content (Borensztajn et al., 1980) might influence their rate of removal. Since it was demonstrated that the chylomicron remnants formed in the cholestatic rat were metabolized in a normal way both in vivo (V) and in vitro (VI), the composition of these remnants was not further investigated. An important practical implication would be that the bile duct ligated rat might be used to prepare large amounts of chylomicron remnants.

The perturbed ultrastructure in the liver cells following bile duct ligation, with disappearance of Golgi complex (Cooper et al., 1974) and a decreased density of lysosomes (Jones et al.,

1976), might be expected to interfere with the process of internalization and degradation of lipoproteins. Since there is no difference between the uptake of chylomicron remnants in different parts of the liver after obstruction of only one of the bile duct branches (V; Cooper and Ockner, 1974), the reduction in Golgi complexes, at least, is of no significance in this context.

Pathological lipoproteins appearing in the cholestatic rat plasma could be expected to compete effectively with the chylomicron remnants for binding to the liver membrane if they, as lipoprotein X in the rat (Felker et al., 1978), contain significant amounts of apoE. In fact, serum from cholestatic animals inhibits the metabolism of chylomicron remnants in hepatocyte cultures more effectively than would be expected from its cholesterol content (VI).

Although estrogen-treatment does not enhance the hepatic remnant uptake in the normal rat (VI) it is possible that in a situation where either the liver mass is restricted, as in porta-caval shunted animals (IV), or the concentration of apoE-containing lipoproteins is significantly increased, as in cholestasis, an apoB,E-receptor mediated uptake of chylomicron remnants might be of significance as a complement to the uptake via the apoE-receptor. Yet, no positive effect on the hepatic remnant uptake is seen in rats treated with estrogen prior to the bile duct ligation (VI). This suggests that the inhibition of the hepatic remnant uptake in the cholestatic state is not mainly caused by a competition by pathological lipoproteins with the remnants for binding to the liver. Also, bile acid infusion causes a significant reduction of the remnant clearance without affecting the plasma lipid composition (VI).

The results obtained in perfusion experiments indicate that the capacity to metabolize chylomicron remnants is reduced in the cholestatic liver even in the absence of serum. This might be the result of a reduced apoE-receptor activity. If this is the case, the regulation of this lipoprotein receptor in the rat differs in an important way from that in the dog, where the apoE-receptor is remarkably stable (Mahley et al., 1981; Angelin et al., 1983). The present studies, however, do not exclude the possibility that a non-specific interference with the endocytotic processes is produced by the changes in the cholestatic plasma.

Regardless of the exact mechanism, it is proposed that the series of events leading to inhibition of hepatic chylomicron remnant uptake in the cholestatic rat includes an initial regurgitation of bile into the blood circulation which in turn leads to a reduction in hepatic clearance of apoE-containing lipoproteins. These will accumulate in plasma and further aggravate the condition by competing with newly arriving chylomicron remnants. An identification of the steps in the lipoprotein binding or the internalization process which are impaired by cholestasis would probably contribute important knowledge to the understanding of receptor-mediated endocytosis in the liver.

CONCLUSIONS

1. The two predominant chylomicron phospholipids, phosphatidylcholine and phosphatidylethanolamine, are metabolized at different rates in the rat, the phosphatidylethanolamine being eliminated from plasma much more rapidly than the phosphatidylcholine. Very early in the process of chylomicron remnant formation, part of both phospholipids can be degraded by lipoprotein lipase, although within a few minutes most of the phospholipids are, together with other polar surface constituents, transferred to HDL. The phosphatidylethanolamine is then rapidly degraded by the hepatic lipase. The removal of chylomicron phosphatidylcholine from HDL also largely occurs via the hepatic lipase, although LCAT contributes to a minor degree.
2. Chylomicron retinyl esters are degraded by an acid retinyl ester hydrolase of rat liver. It is likely that the retinyl ester hydrolysis is catalyzed by the same lysosomal lipase that is able to hydrolyze chylomicron triacylglycerols and cholesteryl esters, thereby degrading all constituents in the non-polar core of the chylomicron remnant.
3. Porta-caval shunt in the rat leads to a marked reduction in liver mass. In spite of this the hepatic uptake of physiological amounts of chylomicron remnants is not significantly impaired, since the uptake of chylomicron remnants per unit liver weight is considerably higher in porta-caval shunted than in sham operated animals. It is suggested that this depends on an increased apoB,E-receptor activity in the livers of porta-caval shunted animals.

4. The uptake of chylomicron remnants in the rat liver is severely reduced after bile duct ligation, whereas the chylomicron remnant formation is not impaired. The hepatic uptake inhibition occurs as a result of the plasma changes induced by bile duct ligation and can be ascribed either to a decreased apoE-receptor activity or to a non-specific interference with the hepatic internalization process. At the present time it is not possible to differentiate between these two mechanisms. The accumulation of lipoproteins in plasma also contributes to the inhibition of chylomicron remnant clearance in the cholestatic rat.

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METABOLISM OF CHYLOMICRON PHOSPHATIDYLETHANOLAMINE IN THE RAT

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Key words: Chylomicron; Phospholipid; Phosphatidylethanolamine; Lipase; (Rat)

Abbreviations: HDL, high-density lipoproteins ($1.063 < d < 1.21$ g/ml); PC, phosphatidylcholine; PE, phosphatidylethanolamine; lysoPC, lysophosphatidylcholine; VLDL, very-high-density lipoproteins ($d < 1.006$ g/ml); Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

SUMMARY

10 minutes after intravenous injection of ^{32}P -labelled chylomicrons 69% of the [^{32}P]phosphatidylethanolamine, but only 34% of the [^{32}P]phosphatidylcholine, was cleared from plasma. Over this time period the plasma phosphatidylethanolamine concentration that was increased after the chylomicron injection returned to normal, indicating that the rapid clearance of [^{32}P]phosphatidylethanolamine was primarily due to metabolism and not due to exchange with tissue phospholipids.

Also after chylomicron [^{32}P]phospholipids had been transferred to high-density lipoproteins in vitro a greater fraction of [^{32}P]phosphatidylethanolamine than of [^{32}P]phosphatidylcholine was eliminated both in the intact rat and in the perfused rat liver. In the latter system the uptake of [^{32}P]phosphatidylethanolamine was reduced by preperfusion with heparin to remove heparin-releasable hepatic lipase.

Experiments with hepatocytes and non-parenchymal liver cells, which both contain only minor amounts of surface-bound hepatic lipase, did not demonstrate any rapid uptake of [^{32}P]phosphatidylethanolamine by exchange with cellular phospholipids.

This study shows that there is a mechanism for rapid clearance of chylomicron phosphatidylethanolamine, which maintains a low plasma phosphatidylethanolamine level (30-40 $\mu\text{g}/\text{ml}$) despite a significant transport of phosphatidylethanolamine with the intestinal lipoproteins. A major part of the rapid phosphatidylethanolamine metabolism occurs in the liver, where the activity of the hepatic lipase is likely to be of crucial importance.

INTRODUCTION

During the intravascular metabolism of chylomicrons a large part of the phospholipid content is transferred to HDL (1,2). This transfer may partly be due to phospholipid exchange but also includes a significant net transfer of phospholipids together with other polar surface compounds, e.g. apolipoprotein A-I (2).

The metabolism of chylomicron phospholipids after their transfer to denser lipoproteins is not yet well understood. Both in vitro and in the perfused rat heart, lipoprotein lipase is active against VLDL or chylomicron PC (3-6), but a significant activity against HDL phospholipids is lacking in vitro (6). Inhibition of hepatic lipase with specific antibodies induces a rapid increase in HDL phospholipids in the rat (7-10), and HDL₂ phospholipids are good substrates for hepatic lipase in vitro (11,12). On the basis of these findings it has been suggested that HDL₂, to which phospholipids have been transferred from the polar surface coat of VLDL and chylomicrons, is a physiological substrate for hepatic lipase.

Chylomicrons contain a higher proportion of PE than other lipoproteins, i.e. about 12% of the chylomicron phospholipids is PE (13), while PE contributes less than 4% to the total plasma phospholipids (14). An efficient clearance from plasma of the PE originating in intestinal lipoproteins might thus be expected. Lecithin:cholesterol acyltransferase is unlikely to be involved in this process, since this enzyme is not active against PE in vitro (15). Hepatic lipase does, however, exhibit a higher activity against PE than against PC in artificial substrates (16), and possibly this is also the case with lipoprotein lipase (17).

This study compares the metabolism of chylomicron PE and PC - present in native chylomicrons or transferred in vitro to HDL - in intact rats, in perfused rat livers and in isolated liver cells.

METHODS

Lipoproteins

^{32}P -labelled chyle was obtained by feeding 0.75 ml of corn oil and 1-2 mCi of sodium [^{32}P]phosphate (Radiochemical Centre, Amersham, Bucks, U.K.) in divided doses during a 6-hr period to rats with a thoracic duct fistula (18). Chyle was collected and stored as described earlier (19). The chyle contained, per ml, 11.5 ± 1.8 mg triacylglycerol, 486 ± 74 μg cholesterol and 94 ± 7 μg lipid phosphorus (means $\pm$ S.E.M., $n=13$). About 90% of the ^{32}P radioactivity was found in the lipid phase ($(3.4 \pm 0.5) \times 10^5$ cpm/ml). $63 \pm 1.2\%$ of this was in [^{32}P]PC, $25\% \pm 0.9\%$ in [^{32}P]PE and $1.6 \pm 0.11\%$ in [^{32}P]lysoPC.

Chylomicrons were isolated according to Minari and Zilversmit (14). $61 \pm 4\%$ of the chyle ^{32}P -labelled phospholipids was in chylomicrons ($S_f > 400$). HDL labelled with ^{32}P -labelled phospholipids was obtained by incubating ^{32}P -labelled chylomicrons with fresh rat serum at 37°C for 1 h during gentle shaking. HDL was then isolated from the mixture by adding an NaCl/KBr solution ($d=1.35$ g/ml) to increase the density to 1.063 g/ml before centrifugation for 36 h at $195,000 \times g$ in an MSE 6 x 14 ml swing-out rotor. The top layer was discarded and the density of the infranatant adjusted to 1.21 g/ml before centrifugation for another 36 h under the same conditions. The resulting top layer containing ^{32}P -labelled HDL was then extensively dialyzed against 0.9% NaCl before use. About one-third of the chylomicron ^{32}P radioactivity was transferred to the HDL when equal amounts of chylomicron and HDL phospholipids were incubated in this way. The

[^{32}P]PC to [^{32}P]PE ratio in the HDL was approx. 50% higher than in the original chylomicrons. Less than 1% of the lipid ^{32}P radioactivity was in lyso[^{32}P]PC.

For studies of in vivo transfer of phospholipids from chylomicrons to denser lipoproteins, blood was collected in chilled tubes containing Na_2EDTA (≈ 1 mg/ml). Plasma was immediately separated and adjusted to $d=1.063$ g/ml. 2.2 ml aliquots of plasma were then layered under 1.1% NaCl ($d=1.006$ g/ml) and centrifuged in an MSE 6x14 ml swing-out rotor at $77,000 \times g$ for 2 h. The tubes were sliced and the top layer (approx. $S_f > 200$) was collected.

Experimental

Male Sprague-Dawley rats (250-300 g) were used throughout the study. In experiments with intact rats the lipoprotein was injected in the left jugular vein during light diethyl ether anaesthesia. The animals were allowed to wake up and were then at the indicated times again anaesthetized and killed by aortic bleeding. Blood was collected in chilled tubes containing Na_2EDTA (≈ 1 mg/ml) and plasma was separated. The livers were rinsed with 20 ml ice-cold saline through the vena cava before extirpation.

Liver perfusions were performed using either a recycling or a non-recycling system. In both cases the livers were initially rinsed with 100 ml Krebs-Ringer buffer before switching to the lipoprotein-containing medium. In the recycling system livers were perfused for 1 h with 50 ml Krebs-Ringer buffer, containing 22% washed human erythrocytes, 3% bovine serum albumin and 0.1% glucose. In the non-recycling system 20 ml Krebs-Ringer buffer was used as medium. All perfusions were performed at 37°C . The flow rate was 15 ml/min. pH was kept at 7.4 by gassing with 95% $\text{O}_2/5\%$ CO_2 . At the end of the experiments the livers were perfused with 50 ml of cold lipoprotein-free Krebs-Ringer buffer.

Hepatocyte monolayer cultures were prepared as described (20). Non-parenchymal liver cells were isolated by centrifugation at $300 \times g$ for 3 min of the supernatant obtained after pelleting the hepatocytes from the mixture of cells prepared by collagenase perfusion. The last contaminating hepatocytes were removed by sedimentation for 10-20 min at 0°C . The non-parenchymal cells were suspended in Hanks' buffer containing 40 mM Hepes, and 2-ml portions were incubated at 37°C during gentle shaking.

Analytical

Lipids were extracted with chloroform/methanol (1:1, v/v). The precipitates were removed and the proportions of chloroform, methanol and water were adjusted to 2:1:1 by adding water and chloroform. The lower phase was washed once with methanol/water/chloroform (48:47:3, v/v) and evaporated. Non-polar lipids were separated by thin-layer chromatography on silica gel G plates in light petroleum/diethyl ether/acetic acid (80:20:1, v/v). Phospholipids were separated on similar plates using chloroform/methanol/acetic acid/water (65:25:4:4, v/v). The spots were visualized with iodine vapour before radioactivity determination.

Lipid phosphorus was determined according to Brante (21). Ethanolamine-containing lipids were determined according to Litman (22), or in later experiments as lipid-soluble fluorescamine-derivatives (23). Aliquots of the lipid extract were then reacted with fluorescamine before two-phase distribution in chloroform/methanol/water (2:1:1, v/v). The lower phase was washed once with methanol/water/chloroform (48:47:3, v/v), taken to dryness with a stream of nitrogen and redissolved in spectroscopically pure ethanol. Fluorescence was then determined in an Aminco-Bowman spectrofluorimeter (excitation wavelength 395 nm, emission wavelength 468 nm). Choline-containing phospholipids were determined enzymatically (Phospholipid B-test, Wako Chemicals, Osaka,

Japan). In the case of chylomicrons and plasma samples that were turbid after the chylomicron injection, the spectrophotometer reading obtained immediately after mixing reagent and sample at 40°C was used as blank value. Cholesterol was determined according to Zak et al. (24) after saponification of the lipid extract (25). Triacylglycerols in lipoproteins were determined by gas-liquid chromatography of the fatty acid methyl esters (26) and in plasma using an enzymatic method (Boehringer Mannheim Scandinavia AB, Bromma, Sweden). Protein was determined according to Lowry et al. (27). Hepatic lipase activity was determined against a [^3H]triolein substrate (28). One unit (U) of enzymatic activity represents the release of 1 μmol fatty acid per min.

RESULTS

Experiments on intact rats

Due to the higher proportion of PE in lymph chylomicrons than in other plasma lipoproteins (14) the plasma PE concentration increased immediately after chylomicron injection (Table I). This increase was, however, rapidly transient and already 10 min after the injection the PE level had returned to its basal range. Thus the PE mass elimination occurred even more rapidly than the clearance of chylomicron triacylglycerols, at least when large amounts of chylomicron lipids were injected (Table I).

To study the transfer of chylomicron phospholipids to denser lipoproteins the $S_f > 200$ plasma lipoprotein fraction was separated. The conditions were chosen to recover most of the chylomicron remnants while still trying to avoid both longer centrifugation times and extensive dilution of the chylomicron remnants with VLDL (S_f 20-400).

The [^{32}P]PC to [^{32}P]PE ratio in the $S_f > 200$ fraction remained close to that in the injected ^{32}P -labelled chylomicrons. Thus the proportion of [^{32}P]PE that may have been lost from the chylomicrons was the same as that of [^{32}P]PC (Fig. 1). Also the same proportions of PC and PE mass disappeared from the chylomicrons early after injection (Fig. 2A).

Less [^{32}P]PE than [^{32}P]PC was, however, recovered in the $S_f < 200$ fraction and also the elimination of [^{32}P]PE occurred more rapidly from this fraction (Fig. 1). About one third of injected PE mass and radioactivity was found in denser particles ($S_f < 200$) 1-5 min after chylomicron injection (Figs. 1 and 2B). Chylomicron remnants contributed very little of this material, since less than 3% of injected [^3H]triacylglycerols was recovered in this fraction after injection of [^3H]palmitic acid-labelled chylomicrons (8.6 mg triacylglycerols).

When HDL, to which chylomicron ^{32}P -labelled phospholipids had been transferred in vitro, was injected into normal rats, the plasma elimination pattern closely resembled that seen after injection of native ^{32}P -labelled chyle or chylomicrons (Figs. 1 and 3). Also the labelling of liver phospholipids was similar in the HDL and in the chylomicron experiments (Fig. 3). After 30 min $45 \pm 2.9\%$ of the [^{32}P]PE and $21 \pm 3.6\%$ of the [^{32}P]PC injected as native chyle was found in the liver, while the corresponding values after HDL injection were $53 \pm 7.0\%$ and $18 \pm 1.7\%$, respectively (means $\pm$ S.E.M., $n=4$). These values are not likely to be influenced by incorporation of contaminating [^{32}P]phosphate into newly synthesized phospholipids. In a control experiment less than 0.2% of the injected ^{32}P -radioactivity was found in liver phospholipids 30 min after intravenous injection of 10 μCi sodium [^{32}P]phosphate in 0.3 ml 0.1 M phosphate buffer.

10 minutes after chylomicron injection $78.5 \pm 3.7\%$ of the $[^{32}\text{P}]\text{PC}$ and $64.5 \pm 4.3\%$ (means $\pm$ SEM, $n=4$) of the $[^{32}\text{P}]\text{PE}$ injected was recovered in liver and plasma together. Thus the $[^{32}\text{P}]\text{PE}$ was subjected to a more extensive hepatic degradation than the $[^{32}\text{P}]\text{PC}$, or the extrahepatic uptake or degradation, i.e. by the action of lipoprotein lipase, was relatively more important for $[^{32}\text{P}]\text{PE}$ than for $[^{32}\text{P}]\text{PC}$. However, the phospholipid recovery after HDL injection was almost identical to that after chylomicron injection. In functionally eviscerated rats, the ratio of $[^{32}\text{P}]\text{PC}$ to $[^{32}\text{P}]\text{PE}$ plasma recovery 10 min after chylomicron injection was reduced from 2.17 ± 0.18 (means $\pm$ S.E.M., $n=18$) in intact rats to 1.38 ± 0.14 ($n=10$) in the eviscerated group. Thus the preference for PE removal was significantly diminished in the absence of a functioning liver. The elimination of $[^{32}\text{P}]\text{PE}$ was inhibited to a similar extent by the evisceration, as were simultaneously injected chylomicron $[^3\text{H}]\text{cholesteryl}$ or $[^3\text{H}]\text{retinyl}$ esters. Heparin injection did not improve the $[^{32}\text{P}]\text{PE}$ removal in the eviscerated rats.

Experiments on perfused rat livers

As in the intact rat, a higher proportion of the added PE than of the PC ^{32}P radioactivity was found in the liver after 1 h perfusion with either ^{32}P -labelled chylomicrons or HDL (Table II). With ^{32}P -labelled chylomicrons there was a rapid initial decrease of both ^{32}P -labelled phospholipids in the medium (Fig. 4), probably due to binding to liver cells or trapping in the sinusoids of whole lipoprotein particles. With both lipoprotein classes $[^{32}\text{P}]\text{PE}$ disappeared at a faster rate than $[^{32}\text{P}]\text{PC}$ from the perfusion medium, the difference being more marked with the HDL than with the chylomicrons (Fig. 4). In short time experiments, when the lipoproteins were not allowed to recirculate through the liver, a preferential liver association of $[^{32}\text{P}]\text{PE}$ was seen only when HDL was used (Table II). The preferential uptake of $[^{32}\text{P}]\text{PE}$ from HDL and also from chylomicrons over longer

periods indicates that some mechanism other than adhesion or uptake of intact lipoprotein particles must be working. Perfusion with heparin-containing buffer before introducing the in vitro labelled HDL reduced the ratio between [32 P]PE and [32 P]PC recovery significantly, although the interindividual variation obscured any possible significant change in the absolute uptake of [32 P]PC or [32 P]PE (Table II). During the heparin perfusion 3.42 ± 0.30 U of hepatic lipase activity was eluted from the livers (12.4 ± 0.63 g, $n=3$), while 0.16 ± 0.02 U could still be measured in homogenates prepared from the livers after perfusion.

Experiments on isolated liver cells

The higher proportion of [32 P]PE than of [32 P]PC taken up in livers in intact rats and in perfusion experiments could be explained by a more extensive hydrolysis of PE by the hepatic lipase followed by an intrahepatic reacylation, or by a more rapid exchange of lipoprotein [32 P]PE with liver membranes, having a higher PE content than lipoproteins. To discriminate further between these possibilities, experiments were performed with collagenase-produced parenchymal and non-parenchymal cells, which both loses virtually all surface-bound hepatic lipase during preparation (29). Addition of heparin to the incubations could, however, considerably increase the hepatic lipase activity in the hepatocyte culture medium (Table III). The increase in enzymatic activity occurred in parallel with a more pronounced preference for [32 P]PE uptake in the hepatocytes (Table III). In the absence of heparin somewhat more of the HDL [32 P]PE than of the [32 P]PC was found associated with the cells, although no such difference was detected using chylomicrons. For example 2.4% of the [32 P]PC and 4.1% of the [32 P]PE was recovered in the hepatocytes after a 4 h incubation with 32 P-labelled HDL (Table III). Non-parenchymal liver cells, which did not accumulate hepatic lipase in the medium, took up very small amounts of phospholipids and did not show any preference for PE uptake (Table III).

DISCUSSION

Although the sources of plasma PE have not been investigated, the difference in the proportions of PE in chylomicron ($\approx 12\%$) and plasma ($< 4\%$) phospholipids is striking (13,14). The PE plasma pool in a 250 g rat could be estimated to approx. 350 μg , and a similar amount of PE would be contributed by the intestinal chyle in 1 h during moderate fat feeding, while only about 15% of the plasma PC and 3% of the plasma apolipoprotein A-I pool is transported with the intestinal chyle during this time (ref. 30, and Landin and Nilsson, unpublished observations). The low plasma concentration of PE is thus not only due to a low rate of secretion into blood but also a mechanism for rapid removal of lipoprotein PE from plasma must exist.

The present study demonstrates that chylomicron PE is actually cleared from plasma very rapidly (Table I, Figs. 1,2 and 3), approx. 70% being cleared during the first 10 min after chylomicron injection. Several metabolic pathways could, however, contribute to this rapid clearance. One possibility is that chylomicron PE is rapidly hydrolyzed by lipoprotein lipase, since this enzyme efficiently hydrolyzes both PC and PE in triacylglycerol-rich lipoproteins in vitro (4). If chylomicron PE was metabolized more quickly than PC by lipoprotein lipase, which might be expected from earlier studies with VLDL (4,17), both the PC to PE mass ratio and the radioactivity ratio would show an increase in the $S_f > 200$ fraction. There was, however, no evidence for such a selective loss of PE from the chylomicrons, but rather the same proportion of PE and PC had been lost at different times 1-6 min after injection (Figs. 1 and 2A). The close parallelity between the removal of $[^{32}\text{P}]\text{PC}$ and $[^{32}\text{P}]\text{PE}$ from the $S_f > 200$ fraction makes the possibility of a rapid hydrolysis of chylomicron PE by lipoprotein lipase combined with an equally rapid transfer of

chylomicron PC to denser lipoprotein particles very unlikely. These data also argue against the possibility of a more sluggish PE than PC transfer from chylomicrons to HDL, which, if occurring, would result in a production of chylomicron remnants enriched in PE, which would be rapidly cleared by the liver.

The second possibility is that the rapid removal of chylomicron PE occurs after the polar surface material has been removed from the chylomicrons. In this case PE, present either in released surface material or in HDL to which polar chylomicron lipids have been transferred, might serve as substrate for hepatic lipase. This enzyme efficiently hydrolyzes PE of HDL (11) and of liposomes (5) in vitro, whereas HDL phospholipids (6) or phospholipids transferred from chylomicrons to HDL in vitro are resistant to lipoprotein lipase (Landin and Nilsson, unpublished observation). Relatively more [^{32}P]PC than [^{32}P]PE was transferred from chylomicrons to HDL during in vitro incubation, probably reflecting a more important exchange of PC than of PE under these conditions (14). However, such HDL ^{32}P -labelled phospholipids were eliminated according to the same pattern as native chylomicron ^{32}P -labelled phospholipids, i.e. the [^{32}P]PE was cleared much more quickly than the [^{32}P]PC (Fig. 3). The finding that the elimination of [^{32}P]PE from the $S_f < 200$ fraction occurred more quickly than that of [^{32}P]PC (Fig. 1) could therefore indicate that chylomicron [^{32}P]PE was metabolized by hepatic lipase after its transfer to denser lipoprotein.

About 50% of the injected [^{32}P]PE radioactivity was found in the liver 30 min after lipoprotein injection, as compared to approx. 20% of the [^{32}P]PC (Fig. 3). These figures are likely to underestimate somewhat the liver uptake since some of the ^{32}P -labelled phospholipids may have been degraded intrahepatically. Further-

more, methylation of [^{32}P]PE could also cause a slight increase in the [^{32}P]PC to [^{32}P]PE ratio in the liver over the longer time intervals studied.

Because it was not possible to follow the disappearance of PE mass in the HDL experiments, data based on ^{32}P radioactivity could obviously be influenced by exchange of plasma phospholipids with phospholipids in liver cells. Liver contains much more PE relative to PC than does the serum, and a more extensive exchange of [^{32}P]PE than of [^{32}P]PC might thus explain the different elimination of the two phospholipids. The data from the experiments with isolated perfused livers and isolated liver cells, however, argue against exchange as a major cause of the rapid [^{32}P]PE removal. Firstly, the rate of uptake of both PC ($\sim 200 \mu\text{g/g}$ per h) and PE ($\sim 90 \mu\text{g/g}$ per h) was comparable in isolated perfused livers (Table II) and livers of intact rats (Fig. 3), despite the 10-fold lower load of phospholipids presented to the livers in the perfusion experiments. Secondly, pretreatment of the perfused livers with heparin diminished the PE uptake compared to the PC uptake (Table II). Heparin also affected the PE uptake from HDL in hepatocyte cultures (Table III). Here the preference for PE uptake varied with the activity of hepatic lipase in the medium in a way that strongly suggests an important role for this enzyme in the rapid hepatic uptake of PE.

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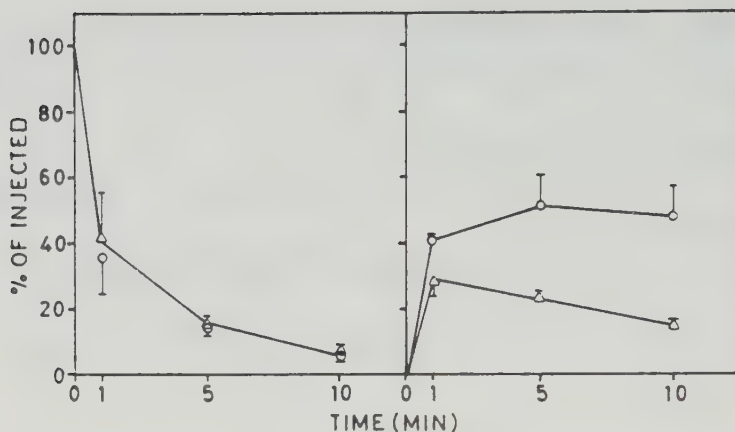


Fig. 1. PC and PE radioactivity in different plasma lipoproteins after injection of ^{32}P -labelled chylomicrons

0.75 ml chylomicrons containing 13.2 mg triacylglycerols and 114 μg lipid phosphorus, 343×10^3 cpm [^{32}P] PC and 128×10^3 cpm [^{32}P] PE was injected. Plasma samples were centrifuged as described in the Methods section. The Figure shows recovery of PC (○) and PE (△) in $S_f > 200$ (left) and $S_f < 200$ (right) fraction. Values are means $\pm$ S.E.M. of three experiments.

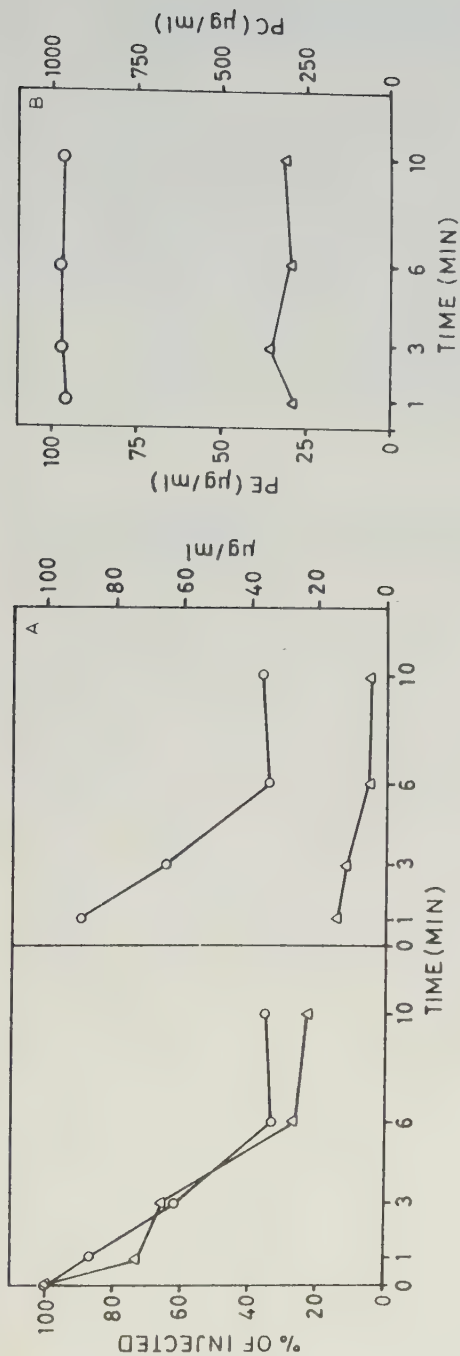


Fig. 2A and B. PC and PE concentrations in different plasma lipoproteins after chylomicron injection. 0.75 ml chylomicrons containing 8.64 mg triacylglycerols, 1350 µg PC and 247 µg PE was injected i.v. At the indicated times the animals were killed by aortic bleeding and chylomicrons and chylomicron remnants were isolated ($S_f > 200$; see Methods section). The recovery of injected PC (○) and PE (△) in the $S_f > 200$ fraction was calculated assuming the phospholipid concentration in this fraction in a rat not recently injected with chylomicrons to be zero (Fig. 2A left). The absolute concentrations of PC (○) and PE (△) in the $S_f > 200$ (Fig. 2A, right) and $S_f < 200$ fractions (Fig. 2B) are also indicated. 30 min after injection the PE concentration was 28 µg/ml in the $S_f < 200$ fraction, but too low to be detected in the $S_f > 200$ fraction. Values are means of duplicate experiments.

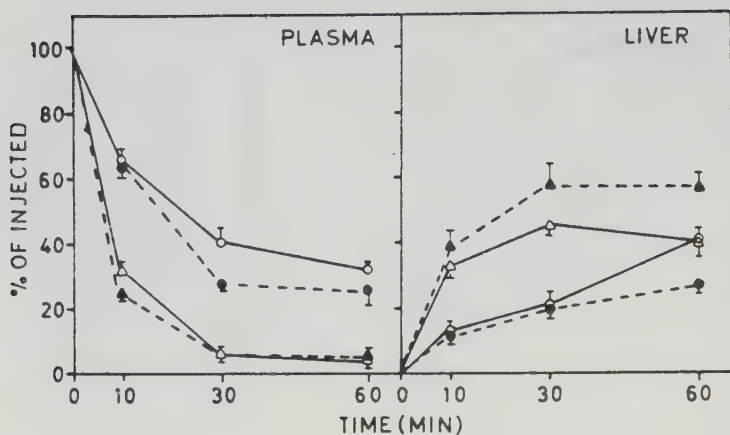


Fig. 3. $[^{32}\text{P}]$ PC and $[^{32}\text{P}]$ PE in plasma and liver after injection of ^{32}P -labelled chyle or HDL

Rats were injected with native ^{32}P -labelled chyle or HDL, isolated from plasma that had been incubated with ^{32}P -labelled chylomicrons in vitro (see Methods). The chyle contained 3.6 mg triacylglycerol, 132 μg cholesterol and 29 μg lipid phosphorus. 61% of the $[^{32}\text{P}]$ phospholipid radioactivity was in PC and 26% in PE. The HDL contained 31 μg lipid phosphorus. 70% of the $[^{32}\text{P}]$ -phospholipid radioactivity was in PC and 16% in PE. The recovery of $[^{32}\text{P}]$ PC (○, ●) and $[^{32}\text{P}]$ PE (△, ▲) was determined in plasma and liver. The plasma volume was assumed to be 4 ml/100 g body weight. Open symbols (○, △) represent experiments with chyle, closed symbols (●, ▲) experiments with HDL. The values indicated are means $\pm$ S.E.M. of 4-6 experiments.

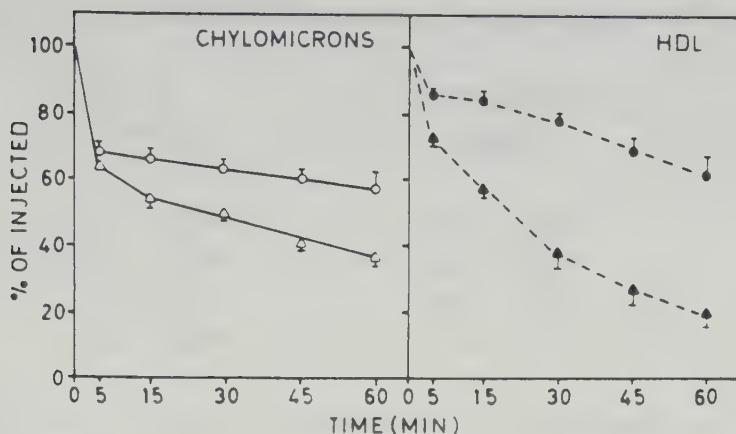


Fig. 4. Elimination of $[^{32}\text{P}]$ PC and $[^{32}\text{P}]$ PE from the medium during rat liver perfusion

Recycling liver perfusions were performed as described in the Methods section and in the legend to Table II. The Fig. demonstrates perfusions with chylomicrons ($\circ, \triangle$) and perfusions with HDL ($\bullet, \blacktriangle$). Aliquots of the medium was removed after different times of perfusion and the percentage of added $[^{32}\text{P}]$ PC ($\circ, \bullet$) and $[^{32}\text{P}]$ PE ($\triangle, \blacktriangle$) remaining in the medium was determined. The liver uptake of $[^{32}\text{P}]$ phospholipids at the end of perfusion is given in Table II. Values are means $\pm$ S.E.M. of 7 experiments with chylomicrons and 4 experiments with HDL.

Table I. Plasma lipids after chylomicron injection.

Time (min)	Triacylglycerols (mg/ml)	PE (μ g/ml)
0	0.56 $\pm$ 0.08	37.7 $\pm$ 2.4
1	2.02 $\pm$ 0.24	56.6 $\pm$ 5.4
5	1.17 $\pm$ 0.25	51.5 $\pm$ 11.4
10	1.15 $\pm$ 0.06	38.2 $\pm$ 3.8

The plasma concentration of triacylglycerols and PE was determined before (time 0) and at different times after intravenous injection of ^{32}P -labelled chylomicrons, containing 22.6 mg triacylglycerols, 262 μ g cholesterol, 2.90 mg PC and 271 μ g PE per injected dose (2 ml). Values are means $\pm$ S.E.M. from four animals at each time point.

Table II. Uptake of [^{32}P]PC and [^{32}P]PE in the perfused rat liver.

	Recovery in liver (% of added dose)		
	[^{32}P]PC	[^{32}P]PE	[^{32}P]PC/[^{32}P]PE
Recycling perfusion			
Chylomicrons (7)	14.4 $\pm$ 3.3**	25.3 $\pm$ 2.8	0.54 $\pm$ 0.09
HDL (4)	10.8 $\pm$ 2.1**	32.8 $\pm$ 4.5	0.33 $\pm$ 0.06
Non-recycling perfusion			
Chylomicrons (8)	24.3 $\pm$ 5.0	21.0 $\pm$ 4.0	1.12 $\pm$ 0.07
HDL (8)	5.5 $\pm$ 0.7**	18.5 $\pm$ 3.7	0.32 $\pm$ 0.03***
HDL after heparin elution (3)	7.2 $\pm$ 0.4*	11.4 $\pm$ 1.2	0.65 $\pm$ 0.09**

The recovery of [^{32}P]PC and [^{32}P]PE and the ratio between these recoveries was determined after a 1 h recycling perfusion or a short-time (1-2 min) non-recycling perfusion with either ^{32}P -labelled chylomicrons or HDL (see Methods section). With the chylomicrons 16 $\pm$ 2.2 mg triacylglycerols, 497 $\pm$ 57 μg cholesterol and 92 $\pm$ 9.2 μg lipid phosphorus and with the HDL 1557 $\pm$ 109 μg cholesterol and 92 $\pm$ 6 μg lipid phosphorus were presented to the livers. In some experiments the livers were perfused with 100 ml heparin-containing buffer (10 U/ml) prior to the lipoprotein-containing medium. Values are means $\pm$ S.E.M. of the number of experiments indicated within brackets. Data were statistically evaluated using Student's t-test.

* $P < 0.05$ ** $P < 0.01$ differences between PC and PE

** $P < 0.01$ *** $P < 0.001$ differences between chylomicrons and HDL

Table III. Uptake of [32 P] PC and [32 P] PE in isolated parenchymal and non-parenchymal liver cells.

Lipoprotein	Time (hrs)	Hepatic lipase (mU/mg)	Uptake in cells (% of added dose)		
			[³² P] PC	[³² P] PE	[³² P] PC/[³² P] PE
Parenchymal cells					
Chylomicrons	2	0.06 ± 0	8.3 ± 1.0	8.4 ± 0.5	0.98 ± 0.07
HDL	2		1.8 ± 0	2.8 ± 0.1	0.65 ± 0.02*
Chylomicrons	4	0.18 ± 0.04	12.7 ± 1.3	13.2 ± 0.8	0.96 ± 0.04
HDL	4		2.4 ± 0.1	4.1 ± 0.1	0.58 ± 0.01**
Parenchymal cells, with heparin					
Chylomicrons	2	0.36 ± 0.05	2.5 ± 0	6.8 ± 0	0.37 ± 0 ⁰⁰
HDL	2		1.7 ± 0	3.7 ± 0.1	0.46 ± 0.01* ⁰
Chylomicrons	4	0.83 ± 0.09	4.3 ± 0.1	11.9 ± 0.4	0.36 ± 0.02 ⁰⁰
HDL	4		2.6 ± 0.1	9.3 ± 0.5	0.28 ± 0.02 ⁰⁰⁰
Nonparenchymal cells					
Chylomicrons	2	0.04 ± 0.02	5.1 ± 0.7	4.8 ± 0.7	1.43 ± 0.43
HDL	2		2.8 ± 0.5	2.8 ± 0.5	0.98 ± 0.03
Chylomicrons	4	0.01 ± 0	4.5 ± 2.0	3.1 ± 0.3	1.38 ± 0.55
HDL	4		2.1 ± 0.3	1.9 ± 0.1	1.11 ± 0.22

Parenchymal liver cells in monolayer cultures (1.8 mg cell protein, 21 μ g lipid phosphorus) were incubated with 2.5 ml serum-free medium containing 32 P-labelled chylomicrons (7.6 μ g lipid phosphorus) or *in vitro* 32 P-labelled HDL (9.0 μ g lipid phosphorus). In some experiments heparin was added simultaneous with the lipoproteins to a final concentration of 50 U/ml medium. Non-parenchymal liver cells (4.9 mg cell protein, 16 μ g lipid phosphorus) were suspended in 2 ml serum-free buffer containing chylomicrons (2.1 μ g lipid phosphorus) or HDL (9.0 μ g lipid phosphorus). The uptake of [32 P] PC and [32 P] PE in the cells as well as the ratio of [32 P] PC to [32 P] PE uptake was determined after 2 and 4 hrs of incubation. In separate incubations the activity of hepatic lipase in the medium was determined and correlated to the cell protein mass. Values are means $\pm$ S.E.M. of 2 to 4 experiments. The [32 P] PC/[32 P] PE ratios were compared using the Student's t-test.

* $p < 0.05$ ** $p < 0.01$ differences between chylomicrons and HDL

⁰ $p < 0.05$ ⁰⁰ $p < 0.01$ ⁰⁰⁰ $p < 0.001$ differences between parenchymal cells with and without heparin.

A ROLE FOR HEPATIC LIPASE IN CHYLOMICRON AND HDL PHOSPHOLIPID METABOLISM

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Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; HDL, high density lipoproteins (d 1.063 - 1.21 g/ml), TG, triacylglycerols.

Supplemented key words. phosphatidylethanolamine • phosphatidylcholine • hepatic lipase antiserum.

Abstract. The rate of removal of phosphatidylethanolamine and phosphatidylcholine from the plasma of rats treated with antiserum to hepatic lipase was measured. The hepatic lipase antiserum was injected intravenously into animals prior to injection of ^{32}P -labeled chylomicrons and high density lipoproteins. Antiserum treatment inhibited removal of ^{32}P -phosphatidylethanolamine from chylomicrons, and the unlabeled serum phosphatidylethanolamine levels increased 2-2.5-fold in 30 min. In contrast, hepatic lipase antiserum had no significant effect on the clearance of chylomicron ^{32}P -phosphatidylcholine or on unlabeled phosphatidylcholine concentrations in serum after injection of chylomicrons.

In experiments in which ^{32}P -labeled high density lipoproteins were injected, the inhibitory effect of the antiserum on the rapid removal of ^{32}P -phosphatidylethanolamine from the circulation was even more marked than its effect on the removal from chylomicrons. The removal of high density lipoprotein phosphatidylcholine on the other hand was unaffected by the antiserum, although a moderate increase in serum phosphatidylcholine concentration was seen.

In antiserum-treated rats injected with ^{32}P -labeled chylomicrons or high density lipoproteins, hepatic ^{32}P -phosphatidylethanolamine radioactivity was decreased. Significantly more ^{32}P -phosphatidylethanolamine was recovered from blood plus liver in the antiserum-treated rats, indicating that the antiserum inhibited the overall degradation of injected ^{32}P -phosphatidylethanolamine. The data suggest that phosphatidylethanolamine is a preferred substrate for hepatic lipase in the metabolism of chylomicron and high density lipoprotein phospholipid.

INTRODUCTION

Although phosphatidylcholine (PC) is the predominant phospholipid in chylomicrons, 10-15% of the phospholipid in these particles is phosphatidylethanolamine (PE) (1). In vitro, lipoprotein lipase can hydrolyze the 1-ester bond of both phospholipids (2,3). During the intravascular metabolism of chylomicrons a significant part of the polar components, including phospholipid, is transferred to high density lipoproteins (HDL) (4,5). These HDL phospholipids are resistant to hydrolysis by lipoprotein lipase (6), but are actively hydrolyzed by hepatic lipase in vitro (7). Recently chylomicron PE was found to be rapidly cleared from plasma, although a significant part was transferred to denser lipoproteins before clearance (8). In view of the high affinity of hepatic lipase for PE in vitro (9), we have considered that the clearance of PE and PC in vivo may be due to the combined action of lipoprotein lipase on chylomicrons and of the hepatic lipase on HDL to which the polar chylomicron surface components have been transferred. To test this hypothesis we investigated the effects of antiserum against hepatic lipase on the metabolism of chylomicron and HDL phosphatidylethanolamine and phosphatidylcholine in vivo.

METHODS

^{32}P -labeled chyle was obtained by feeding 2 mCi sodium ^{32}P -phosphate (3-7 mCi/mg P) (Amersham) and 0.5 ml corn oil to rats with thoracic duct drainage (10). Chylomicrons were isolated by adjusting the density of the lymph to 1.063 g/ml with KBr/NaCl ($d=1.35$ g/ml), layering under saline ($d=1.006$ g/ml) and then centrifuging at average 78,000 X g for 120 min. The chylomicrons thus obtained were suspended in 0.15 M NaCl.

^{32}P -labeled HDL was obtained by incubating 5 ml ^{32}P -labeled chylomicrons, containing 46 mg TG, 5.5 mg PC and 1.5 mg PE (Expt. A) or 162 mg TG, 10.1 mg PC and 0.88 mg PE (Expt. B), with 10 ml fresh rat serum for 2 hr at 37°C . Lipoproteins with $d < 1.063 \text{ g/ml}$ were removed by centrifuging the mixture adjusted to this density at average $195,000 \times g$ for 36 hr before isolating the HDL by recentrifuging at $d = 1.21 \text{ g/ml}$ at the same speed for another 36 hr. The top fraction obtained was dialyzed against 0.15 M NaCl before use. All centrifugations were performed in an MSE 6 X 14 swing-out rotor at 10°C .

The purification of hepatic lipase and preparation of antiserum to the pure protein have been described (11). Briefly, rat liver hepatic lipase purified about 60,000-fold to a specific activity of 30,000 $\mu\text{moles FFA/h/mg}$ protein against a triolein substrate was used to raise antibody in a rabbit. This antiserum completely inhibited the hepatic lipase activity in the supernatant fraction of liver homogenates and also the enzyme activity released from liver by perfusion with heparin (11). Antibody titer was calculated from its inhibition of pure hepatic lipase activity with an emulsified triglyceride substrate; 60 μl antiserum was able to inactivate all the hepatic lipase activity from one gram of liver tissue, approximately 1 Unit of lipase activity. A Unit of enzyme activity is defined as the release of 1 $\mu\text{mole FFA/h/mg}$ protein from an emulsified triglyceride substrate (11). Since only the fraction of hepatic lipase on the cell surfaces would be inactivated by circulating antibody, the antiserum dose, 200-250 μl , was calculated to be in considerable excess. Monospecificity of the antiserum was evidenced by the observation that, using a hepatic lipase antiserum immunoabsorbent column, a single band was obtained from liver extracts on SDS electrophoresis (11).

Male Sprague-Dawley rats, weighing 100-150 g, fasted overnight, were used. Rabbit antiserum to rat hepatic lipase or an equivalent volume of serum from a nonimmunized rabbit was injected intravenously 5 min before the injection of 0.3 ml ^{32}P -labelled chylomicrons or 0.5 ml ^{32}P -labelled HDL. Thirty min after the lipoprotein injection the animals were killed by aortic bleeding and serum collected. Two series of experiments using different batches of ^{32}P -labelled chyle were performed. In experiment A 250 μl antiserum or control serum was injected as described above followed by chylomicrons containing 2.8 mg TG, 0.33 mg PC (1.18×10^6 cpm) and 92 μg PE (2.44×10^5 cpm) or HDL containing 0.33 mg PC (4.9×10^5 cpm) and 51 μg PE (3.83×10^4 cpm). The animals were kept under light ether anesthesia and blood collected from the tail into tared test tubes at specified intervals. At the end of the experiment, i.e. 30 min after the lipoprotein injection, the animals were killed as described above. In experiment B 200 μl antiserum or control serum was injected followed by chylomicrons containing 9.7 mg TG, 0.61 mg PC (5.38×10^5 cpm) and 53 μg PE (2.00×10^5 cpm) or HDL containing 0.28 mg PC (9.09×10^4 cpm) and 11 μg PE (1.86×10^4 cpm). In this case the animals were allowed to wake up after the injection and were again anesthetized immediately before the aortic puncture.

Triacylglycerols were estimated by an enzymatic method (Boehringer-Mannheim GmbH) and choline-phosphatides by a phospholipase-choline oxidase method (Phospholipids B-test, Wako Chemicals, Osaka, Japan). PE was determined by fluorimetry after conversion to its fluorescamine derivative. The lipid extract from 50 μl serum was reacted with fluorescamine according to Schmid et al. (12). The reaction was terminated by adding 2 ml chloroform, 1.5 ml water, and 1.5 ml methanol. The upper phase was discarded and the lower phase washed twice with methanol/water/chloroform (48:47:3). Then the chloroform phase was taken to dryness and redissolved in spectroscopically pure ethanol.

Fluorescence was determined in an Aminco Bowman spectrophoto-fluorimeter (excitation wavelength 395 nm, emission wavelength 468 nm). Similar results were obtained when the reaction mixture was subjected to thin layer chromatography and the fluorescence of the dominant spot seen under UV-light, i.e. the PE fluoresc-amine derivative, was determined after extraction according to Schmid et al. (12).

Lipids in blood, serum and liver samples were extracted with chloroform/methanol (1:1) and the extracts washed as described earlier (10). The lipid extracts were dried under nitrogen, redissolved in chloroform and subjected to thin layer chromatography on silica gel G plates developed in chloroform:methanol:water:acetic acid (65:25:4:4). The PC and PE spots were scraped into counting vials; 1 ml methanol/ water (1:1) was added and the vessels were vortexed before adding 10 ml toluol:Instagel (Packard Instr. Inc.) (1:1). Radioactivity was determined in a Packard TriCarb Liquid Scintillator. The data are expressed as % injected dose. The total blood weight, calculated as 8% of total body weight was 9.2 - 10.9 g (mean, 10 g). The total serum volume was calculated as 4% of body weight.

Values presented are the means $\pm$ SEM for the number of animals indicated. Statistical evaluation of the data was performed using the Student's t-test.

RESULTS

Figure 1 shows the marked difference in the rate of disappearance of chylomicron ^{32}P -PE and ^{32}P -PC from the plasma of the control rats. A large part of both ^{32}P -PC and ^{32}P -PE, 40% and 60% respectively, was eliminated rapidly within the first 2 minutes; thereafter the clearance of ^{32}P -PC was notably slower than that of ^{32}P -PE.

The antiserum against hepatic lipase caused inhibition of chylomicron ^{32}P -PE clearance at all time intervals (Fig. 1). However, even in the presence of antiserum to hepatic lipase, a significant part of both ^{32}P -labeled chylomicron phospholipids was still cleared during the first 5 minutes. The clearance of ^{32}P -PE was thereafter significantly inhibited, and at 30 min 25-30% remained in the plasma of antiserum treated rats as compared to 2.5% in the control animals (Table I). The effect of the antiserum on chylomicron ^{32}P -PC clearance was much less marked than in case of ^{32}P -PE. Indeed, there was no significant inhibition of plasma ^{32}P -PC clearance at any time interval (Fig. 1, Table I).

When ^{32}P -labeled HDL was injected the initial removal of ^{32}P -PE was less rapid than that observed with labeled chylomicrons (Fig. 2). But still, in the control animals the clearance of ^{32}P -PE exceeded 80% in 30 min. The inhibition of the ^{32}P -PE removal by hepatic lipase antiserum was very marked, with 50-60% remaining in blood after 30 min. In contrast, there was again no significant inhibition by the antiserum of the ^{32}P -PC removal (Fig. 2, Table I).

The plasma PE concentrations were markedly increased 30 min after the injection of lipoproteins into animals pretreated with antiserum (Table II). The rise in serum PE concentration exceeded the amounts which could possibly have been contributed by the injected lipoproteins. This is very clear since the total PE contained in the injected lipoproteins ranged from 10-90 μg and the mean increases in the total serum PE amounted to almost 200 μg . A significant increase in serum PC was seen only after HDL injection while a considerable degree of variation between individuals obscured any possible difference between the antiserum-treated and control group after chylomicron injection. However, the increase in serum PC caused by the antiserum also

exceeded the amount contributed by the injected lipoproteins. There were no differences in plasma triacylglycerol levels between the different groups (Table II), indicating that the antiserum had no significant effect on the clearance of either chylomicron or HDL triacylglycerols.

In rats injected with either chylomicrons or HDL the hepatic ^{32}P -PE radioactivity in rats injected with antiserum was lower than in the control rats (Table I) although the differences were not as pronounced as the marked differences in removal of ^{32}P -PE from plasma. The recovery of ^{32}P -PE from blood plus liver was higher in the antiserum treated groups (Table I), indicating that the proportion of overall ^{32}P -PE degradation was less in these groups. The average recovery of ^{32}P -PE after injection of chylomicrons plus antiserum was lower than after injection of HDL plus antiserum, indicating that a higher proportion of ^{32}P -PE in chylomicrons than in HDL was catabolized in some way unaffected by hepatic lipase antiserum, probably by lipoprotein lipase. The differences in total recovery of ^{32}P -PC between antiserum treated and control animals were not significant (Table I).

DISCUSSION

The present study indicates that hepatic lipase has an important role in the metabolism of chylomicron and HDL PE. Hepatic lipase antiserum significantly decrease both the amount of ^{32}P -PE eliminated from serum and that recovered in the liver 30 min after lipoprotein injection (Table I). During the same time period no significant effect of the antiserum on the metabolism of chylomicron or HDL ^{32}P -PC could be detected. The normal elimination rate of chylomicron PC is, however, much slower than that of PE and moderate changes in the clearance of PC would thus

be more difficult to detect. Further experiments over longer time periods will be needed for a quantitative estimation of the amount of chylomicron PC that is metabolized by hepatic lipase.

Administration of hepatic lipase antiserum not only inhibited the clearance of chylomicron or HDL ^{32}P -PE, but also caused the serum level of unlabeled PE to increase more than 100% which is far more than could be accounted for by the injected PE. A significant increase in unlabeled PC concentration was detected only in animals injected with antiserum and HDL, and this increase amounted to only about 15%. This indicates further that serum PE turns over more rapidly, perhaps up to 10 times faster, than serum PC. The role of PE in the transport of phospholipid fatty acids may thus be more important than is apparent from the low plasma PE concentration.

In experiments where chylomicrons were injected there was a rapid initial clearance of a part of both PE and PC, which cannot be inhibited by hepatic lipase antiserum (Fig. 1). In our earlier experiments (8) there was also a rapid initial disappearance of both PE and PC from the injected chylomicrons, which exceeded the appearance of these phospholipids in denser lipoproteins. It is likely that the effect of lipoprotein lipase on chylomicrons contributes to this initial disappearance. The proportion of chylomicron phospholipids that is metabolized by the lipoprotein lipase pathway is difficult to determine quantitatively, but a rough estimation from the initial slope of the curves for PE and PC clearance from the antiserum treated animals (Fig. 1) indicates that it could amount to about 40% of the injected PE and 20-30% of the PC. It should be noted that hepatic lipase antiserum inhibits chylomicron PE clearance at a time when the substrate for the enzyme could be phospholipids in either chylomicrons, released chylomicron surface material which has not been integrated into HDL particles, or HDL particles to which chylo-

micron phospholipids have been transferred. Although the data indicate an important role for hepatic lipase in the metabolism of chylomicron phospholipids, they do not provide information as to which particles are the physiological substrate for the enzyme. Our earlier data (8) indicate, however, that the transfer of remaining phospholipids to denser lipoproteins during the metabolism of chylomicrons is very rapid, and it is extremely likely that the data obtained 5-30 min after injection represent the metabolism of phospholipids in dense lipoproteins, presumably HDL. The findings in the present study are thus compatible with the idea that phospholipid-rich HDL is the preferred substrate for hepatic lipase (7,13-16).

A decrease in hepatic ^{32}P -PE radioactivity was seen in the antiserum-treated rats, but it was less than expected from the marked effects of antiserum on removal of ^{32}P -PE from the plasma. However, the total recovery of PE radioactivity in serum plus liver was larger in the rats that had been treated with antiserum than in the controls. It is thus possible that most of the hepatic ^{32}P -PE in the controls had been formed in a deacylation-reacylation cycle of chylomicron PE, during which a significant part of the ^{32}P -2-lyso-PE formed had been degraded further rather than reacylated. In contrast, in the experiments with antiserum more ^{32}P -PE was available in plasma for exchange reactions with liver phospholipids.

The finding that PE hydrolysis is greatly inhibited by the hepatic lipase antiserum whereas PC is virtually unaffected suggests that PE is a specific substrate for the hepatic lipase. This is in line with earlier findings by Ehnholm et al. (9) who noted that the hepatic lipase activity in human post heparin plasma was approximately 25-fold greater with PE as substrate than with PC.

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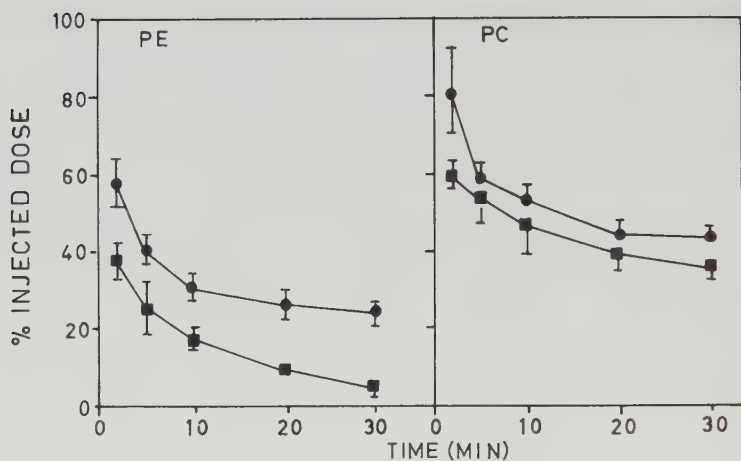


Fig. 1. Effect of hepatic lipase antiserum on clearance of chylomicron ^{32}P -phosphatidylethanolamine and ^{32}P -phosphatidylcholine. Rats were injected with antiserum against hepatic lipase (●—●) or control serum (■—■) 5 min prior to the chylomicron administration as described for expt A in the Methods section. The animals were kept under light ether anesthesia and blood sampled from the tail at specified intervals. Values are the means $\pm$ SEM for 3 animals.

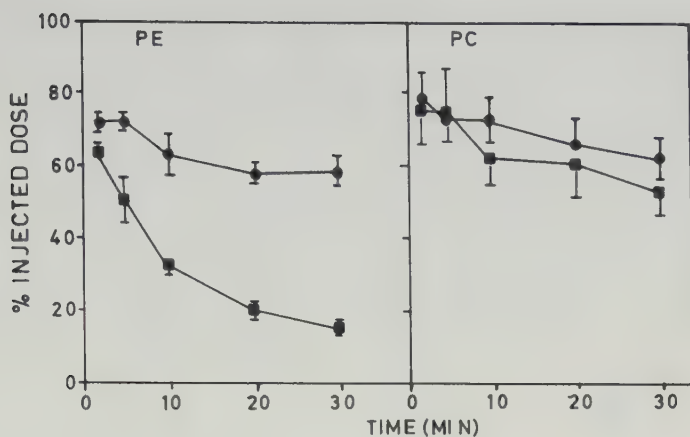


Fig. 2. Effect of hepatic lipase antiserum on clearance of HDL ^{32}P -phosphatidylethanolamine and ^{32}P -phosphatidylcholine. Animals were treated as described in the Methods section (Expt A) and in the legend to Fig. 1. Values are the means $\pm$ SEM for 3 animals.

Table I. Effect of hepatic lipase antiserum on tissue distribution of ^{32}P -labeled phosphatidylcholine and phosphatidylethanolamine.

Organ	Anti-serum	% of Injected Radioactivity			
		Chylomicrons		HDL	
		PC	PE	PC	PE
Serum/blood	+	57.0 $\pm$ 3.1	27.5 $\pm$ 1.8***	58.3 $\pm$ 2.5	52.2 $\pm$ 3.3***
	-	52.3 $\pm$ 4.6	11.3 $\pm$ 2.0	53.6 $\pm$ 2.5	18.3 $\pm$ 1.0
	+	12.6 $\pm$ 0.4	27.7 $\pm$ 2.3**	12.0 $\pm$ 0.7	27.1 $\pm$ 2.1**
	-	14.3 $\pm$ 0.8	37.2 $\pm$ 2.1	10.9 $\pm$ 0.3	43.9 $\pm$ 1.9
Serum/blood+ liver	+	69.7 $\pm$ 2.9	55.3 $\pm$ 1.8*	70.4 $\pm$ 2.6	79.3 $\pm$ 4.0**
	-	66.6 $\pm$ 4.2	48.5 $\pm$ 1.8	64.5 $\pm$ 2.4	62.2 $\pm$ 1.7

Tissues were obtained from animals treated as described in the Methods Section 30 min after the lipoprotein injection. Values are means $\pm$ SEM for the 9 animals of experiments A and B.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table II. Effect of hepatic lipase antiserum on serum lipid levels of animals injected with lipoprotein fractions.

Lipoprotein	Anti-serum	TG (mg/ml)	PC (mg/ml)	PE (μ g/ml)
Chylomicrons	+	1.11 ± 0.15	1.68 ± 0.07	$65.8 \pm 6.7^{***}$
	-	0.99 ± 0.15	1.59 ± 0.09	28.5 ± 2.7
HDL	+	0.70 ± 0.06	$1.53 \pm 0.04^{**}$	$68.4 \pm 4.8^{***}$
	-	0.59 ± 0.04	1.34 ± 0.04	28.1 ± 2.1

Serum was obtained 30 min after lipoprotein injection. The experiments are described in the Methods Section. Values are means $\pm$ SEM for the 9 animals of experiments A and B.

** $p < 0.01$

*** $p < 0.001$

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HYDROLYSIS OF CHYLOMICRON RETINYL ESTERS BY AN ACID HYDROLASE OF RAT LIVER

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Rat liver homogenate hydrolyzed chylomicron remnant retinyl esters with pH optima at pH 4.5, 6 and 8. The retinyl ester hydrolysis at pH 4.5 was correlated to the acid phosphatase activity in different subcellular fractions, and thus the highest activity was found in highly purified lysosomes. These fractions could also catalyze the reverse reaction, i.e., the esterification of retinol when incubated with excess palmitic acid. Purified rat liver lysosomes hydrolyzed chylomicron retinyl and cholesteryl esters with comparable rates, both being much lower than the hydrolysis of chylomicron triacylglycerols.

Dietary retinol is transported, mainly in esterified form, with the chylomicrons from the intestine [1]. During the intravascular hydrolysis of most of the chylomicron triacylglycerol [2] the retinyl esters are retained in the chylomicron remnants, and thus rapidly accumulate in the liver [1]. The chylomicron remnants are assumed to be taken up in the hepatocytes [3] by a receptor-mediated endocytosis [4–6]. The chylomicron remnant cholesteryl ester [6] and protein [7] portions are then degraded in the lysosomes. Since these processes are thought to reflect the degradation of the entire chylomicron remnant particle, a similar degradation of the retinyl esters would also be expected. Evidence exists for an initial hydrolysis followed by a re-esterification of the retinyl esters of dietary origin [1,8], preceding their ultimate storage in the fat-storing cells in the liver [9,10]. A lysosomal cholesteryl ester hydrolase able to degrade chylomicron remnants in the rat liver has been demonstrated previously in this laboratory [11]. However, reports in the literature on retinyl ester-hydrolyzing activities in the rat liver [12–15] have focused on hydrolysis around pH 8. This

study was undertaken to investigate the hydrolysis of retinyl esters in rat liver homogenates and purified liver lysosomes at low pH and using a physiological substrate, such as [³H]retinyl esters in chylomicron remnants.

Male Sprague-Dawley rats, weighing 225–275 g, were fasted overnight and then killed by aortic bleeding under diethyl ether anesthesia. The livers were rinsed with ice-cold saline through the vena cava and immediately homogenized in cold 0.25 M sucrose. After filtering through nylon gauze the homogenate was subjected to subcellular fractionation following the schedule of Ragab et al. [16]. The 'light mitochondrial pellet' (11 700 × g pellet) thus obtained was further subfractionated in a metrizamide gradient [17]. The bands obtained in the gradient were named A ($d < 1.109$), B ($1.109 < d < 1.135$), C ($1.135 < d < 1.145$) and D ($1.145 < d < 1.181$). Aliquots of the homogenate and the fractions obtained during the different centrifugation steps were stored at -20°C until analyzed. Protein was determined using the method of Lowry et al. [18] after precipitation with 10% trichloroacetic acid [17]. Acid phosphatase was

determined by incubating 25–100- μ l aliquots of the diluted homogenate or subcellular fractions with 0.5 ml β -glycerophosphate (10 mg/ml) in 0.1 M acetate buffer, pH 5.0. After incubation for 1 h at 37°C the incubations were stopped by adding ice-cold 10% trichloroacetic acid/2% ascorbic acid to a final volume of 2 ml. After centrifugation, the amount of phosphorus liberated was determined in the supernatants. 500- μ l aliquots of the supernatants were diluted with an equal volume of distilled water. 0.5 ml 1% ammonium molybdate was added, followed by 1 ml of a reagent containing 20 mg sodium citrate and 20 mg sodium arsenite in 2% acetic acid. After 15 min at room temperature the phosphorus content was determined by measuring the absorbance at 700 nm and comparing with a standard curve ranging from 5 to 75 nmol phosphate obtained by using a KH_2PO_4 stock solution. All determinations of acid phosphatase activity were corrected for the basal content of inorganic phosphorus in the samples before incubation. 1 unit (U) of acid phosphatase was defined as the liberation of 1 nmol of phosphorus per h. All enzyme activities were correlated to the protein content.

Radioactively labelled chyle was obtained by feeding 200 μCi [^3H]retinol (New England Nuclear, Dreieich, F.R.G.) or 125 μCi [^{14}C]linoleic acid and 250 μCi [^3H]cholesterol (Radiochemical Centre, Amersham, U.K.) in corn oil to lymph fistula rats as described previously [19]. Chylomicron remnants were prepared *in vitro* by incubation with post-heparin plasma [20]. Retinol was determined by high-performance liquid chromatography on Nucleosil C_{18} using water/methanol (25 : 75, v/v) as the mobile phase. Retinyl esters were degraded by alkaline hydrolysis prior to the determinations. 13-*cis* retinol was used as an internal standard. All samples containing [^3H]retinol were handled in dim light and extracted and stored in solvents containing 0.05% (w/v) butylated hydroxytoluene after adding retinol and retinyl ester carrier. Cholesterol and triacylglycerols were determined using enzymatic methods (Boehringer Mannheim GmbH, F.R.G.). The extraction, separation by thin-layer chromatography and determination of radioactivity in the different lipid fractions has been described previously [19]. Incubations for determination of

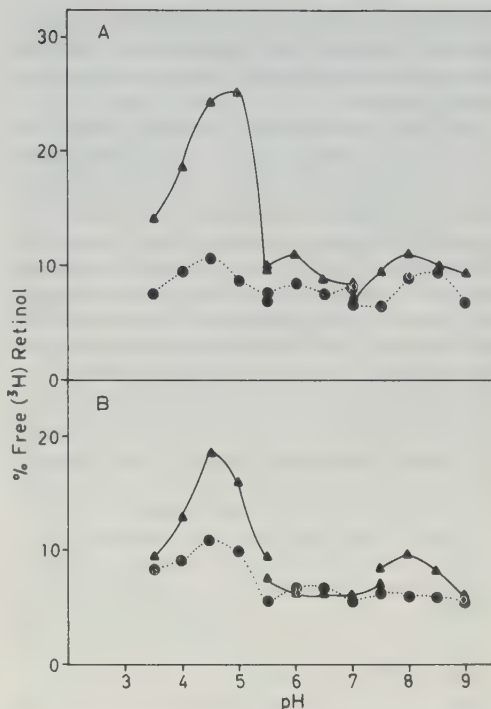


Fig. 1. Hydrolysis of chylomicron [^3H]retinyl esters in rat liver homogenates. Rats were injected with native [^3H]retinol-labelled chyle containing, per ml, 8.3 mg triacylglycerols, 434 μg cholesterol, 55 μg retinol, $2.61 \cdot 10^6$ cpm ^3H , of which 95.1% was in esterified retinol and 2.3% in free retinol. 20 min after the injection the rats were killed by aortic bleeding, the livers rinsed with ice-cold saline and a 10% (w/v) liver homogenate was prepared in 0.25 M sucrose/10 mM mercaptoethanol/1 mM Na_2EDTA . After filtering through nylon gauze, 200- μ l aliquots of the homogenates were incubated with 400 μ l of different buffers for 30 ($\bullet$) or 120 (Δ) min. In experiment A (upper panel) one rat was injected with 0.5 ml of the [^3H]retinol-labelled chyle. The buffers used were 0.1 M Tris-acetate (pH 3.5–5.5), 0.1 M Tris-maleate (pH 5.5–7.0) and 0.1 M Tris-HCl (pH 7.0–9.0). In experiment B (lower panel) one rat was injected with 1 ml [^3H]retinol-labelled chyle. The buffers used were 0.1 M acetate (pH 3.5–5.5), 0.1 M phosphate (pH 5.5–7.5) and 0.1 M Tris-HCl (pH 7.5–9.0).

hydrolyzing or esterifying activity were, when not otherwise stated, performed for 30 min at 37°C during gentle shaking. The reactions were stopped by adding cold chloroform/methanol (1 : 1, v/v) containing carrier and anti-oxidant as described

above. All incubations were performed in duplicate and the results were corrected for the values obtained in blank incubations without any enzyme source present.

Fig. 1 demonstrates the hydrolysis of [^3H]retinyl esters at different pH in liver homogenates from rats that had received an intravenous injection of [^3H]retinol-labelled chyle 20 min before the preparation of the homogenate. An extensive hydrolysis was found at pH 4.5 and this pH optimum was further accentuated by extending the incubation time. Hydrolysis was also seen at pH 6.0–6.5 and at pH 8.0, the pattern thus roughly corresponding to the degradation of chylomicron remnant cholesteryl esters in rat liver homogenate [11]. Although this kind of experiment does not allow any comparison between the importance of

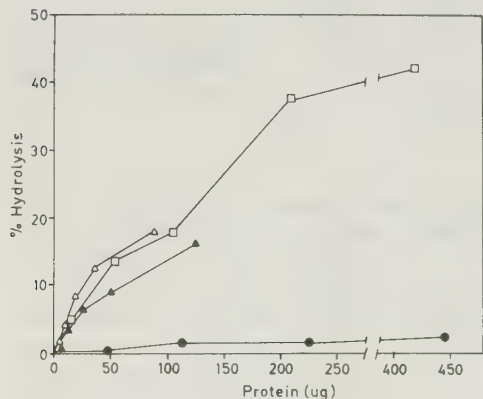


Fig. 2. Hydrolysis of chylomicron remnant [^3H]retinyl esters with rat liver homogenate and purified rat liver lysosomes. Chylomicron remnants (80 pmol retinyl esters, 1637 cpm ^3H in retinyl esters and 32 cpm ^3H in retinol) were incubated with liver homogenate (●), containing 416 U acid phosphatase per mg protein, in a total volume of 1.75 ml 0.06 M acetate buffer, pH 4.5. Two separate preparations of purified lysosomes were incubated at the same conditions. One preparation (▲), 27-fold enriched in acid phosphatase activity (14830 U/mg), originated from the homogenate included in the figure, while the other (△), 36-fold enriched in acid phosphatase (20700 U/mg), was prepared from another rat liver. The figure also shows one experiment where chylomicron remnants (447 pmol retinyl esters, 2479 cpm ^3H in retinyl esters and 175 cpm in retinol) were incubated with purified lysosomes (□), 96-fold enriched in acid phosphatase activity (43100 U/mg), in a total volume of 9.2 ml 0.05 M acetate buffer, pH 4.5, also containing 0.1% defatted bovine serum albumin.

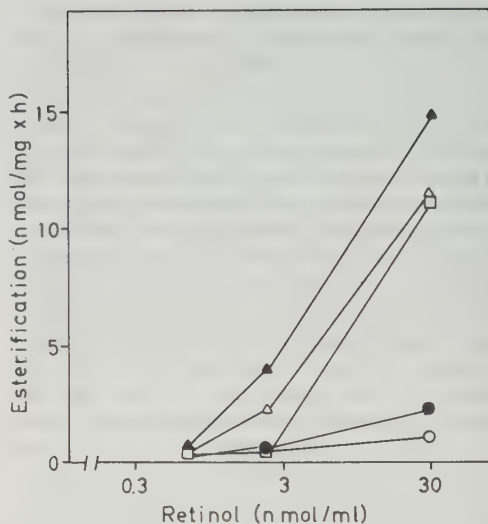


Fig. 3. Esterification of retinol with liver homogenate and purified lysosomes. Fractions from separate rat livers were incubated with [^3H]retinol at 0.64, 2.4 and 30 nmol/ml for 30 min at 37°C. The incubation conditions were: 0.64 nmol/ml: 0.7 nmol retinol, 5500 cpm ^3H , 85–2320 µg protein, 14 mg palmitic acid, 9 mg defatted bovine serum albumin, 1.1 ml total volume; 2.4 nmol/ml: 7.0 nmol retinol, 13266 cpm ^3H , 35–625 µg protein, 1.5 mg palmitic acid, 250 mg bovine serum albumin, 3.0 ml; 30 nmol/ml: 30 nmol retinol, 32076 cpm ^3H , 24–667 µg protein, 1.5 mg palmitic acid, 250 mg bovine serum albumin, 1.0 ml. The fractions used were: homogenate (○), 11700× g pellet (●), purified lysosomes A (△), B (▲) and C (□). The 11700× g pellet was 5-fold enriched in acid phosphatase while the corresponding enrichment in the most highly purified lysosomes (B, ▲) was 96-fold.

the different degradation pathways for retinyl esters in liver, it strongly suggests the existence of a lysosomal retinyl ester hydrolase in rat liver, apart from the rat liver retinyl ester hydrolase with pH optimum around 8 which has been described previously [12,13,22]. It is, however, notable that earlier investigators have in no case extended the pH curves lower than pH 5.

The hydrolysis of chylomicron remnant [^3H]retinyl esters when incubated *in vitro* at pH 4.5 with liver homogenate was very slow. Purified lysosomes, however, were able to hydrolyze the retinyl esters at a significant rate (Fig. 2); e.g., at a retinyl ester concentration of about 40 pmol/ml the homogenate hydrolyzed 0.02 nmol/mg per h while

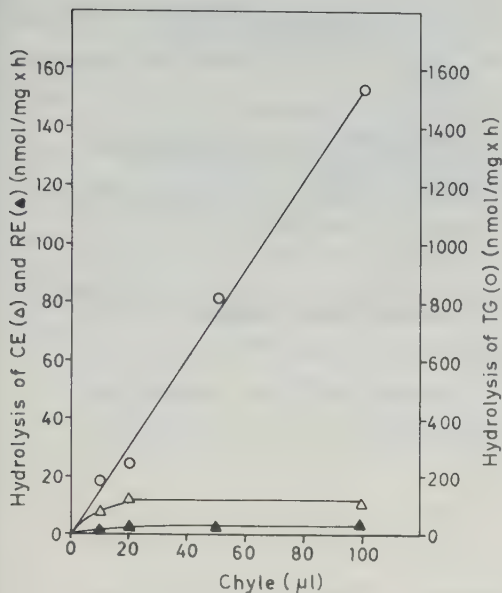


Fig. 4. Hydrolysis of chyle by purified lysosomes. Lysosomes (7.8 μ g protein, 348 U acid phosphatase, 36-fold enriched in acid phosphatase compared to the starting homogenate) were incubated in parallel with either [3 H]retinol-labelled or [3 H]cholesterol/[14 C]linoleic acid-labelled native chyle in a total volume of 200 μ l 0.05 M K_2HPO_4 /0.025 M citrate/4% (w/v) bovine serum albumin, pH 4.0 for 2 h. The different chyles contained per 100 μ l: 1900 nmol triacylglycerols (64 154 cpm 14 C) and 70 nmol cholesteryl esters (63 228 cpm 3 H) or 4.2 nmol retinyl esters (22 155 cpm 3 H), 2389 nmol triacylglycerols and 68 nmol cholesteryl esters, respectively. The hydrolysis of triacylglycerols (TG, ○) was 1532, cholesteryl esters (CE, Δ) 10.8 and retinyl esters (RE, ▲) 3.8 nmol/mg per h when 100 μ l chyle was added.

different preparations of lysosomes hydrolyzed 0.21, 0.33 and 1.5 nmol/mg per h (calculated at the points between 88 and 125 μ g protein indicated in Fig. 2). The rate of hydrolysis was stable for incubation times up to at least 4 h (data not shown).

Liver homogenates from different rats varied considerably in their retinyl ester-hydrolyzing activity (e.g., 0.01, 0.06, 0.13, 0.62 nmol/mg per h at a retinyl ester concentration of 265 pmol/ml). Apart from the possible influence on these figures by using different preparations of chylomicron remnants, which might differ in size as well as in

lipid and protein composition, it is interesting to note that a more than 50-fold individual variation has earlier been described by Prystowsky et al. [14] for the retinyl palmitate hydrolysis at pH 8.0 by rat liver homogenates. However, crude or purified lysosomal preparations always hydrolyzed retinyl esters at a higher rate than did the original liver homogenate. Furthermore, the acid retinyl ester hydrolysis varied in a way similar to the acid phosphatase activity in the different fractions obtained during the lysosome purification.

The subcellular fractions able to hydrolyze chylomicron remnant retinyl esters were also able to esterify retinol when incubated with retinol and excess palmitic acid (Fig. 3). Also in this case the more purified lysosomes were more effective than the 11 700 $\times$ g pellet or the liver homogenate. A similar reversibility has earlier been described for the acid cholesteryl esterase of rat liver [11,23].

The purified lysosomes were able to hydrolyze both chylomicron triacylglycerols at a very high rate and also cholesteryl and retinyl esters at much lower but similar rates (Fig. 4). It is possible that the retinyl ester hydrolase described in this study is identical to the lysosomal cholesteryl ester hydrolase that has been shown previously to degrade both chylomicron triacylglycerols and cholesteryl esters [11]. Also lysosomal lipase which has been purified from rat [24] and human [25] livers can attack both triacylglycerols and cholesteryl esters in artificial substrates. It is thus probable that all nonpolar components of the chylomicron remnants are degraded by the action of one common lysosomal enzyme in the liver.

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EFFICIENT HEPATIC UPTAKE OF CHYLOMICRON REMNANT CHOLESTEROL
IN RATS WITH A PORTA-CAVAL ANASTOMOSIS

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Short title: Chylomicron metabolism after porta-caval shunt

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Key words. Porta-caval shunt, chylomicron remnant, lipoprotein
lipase, lipid uptake.

ABSTRACT

Porta-caval shunted and sham operated male rats, fed ad libitum and being of similar weight, were studied 2-3 weeks after surgery. At this time serum cholesterol did not differ significantly between the two groups, while serum triacylglycerols and phospholipids were lower in the shunted group. These animals also showed an increased serum bile acid level and an increased serum estradiol/testosterone ratio. The metabolism of native chyle labelled with [^3H]cholesterol and [^{14}C]linoleic acid, or preformed chylomicron remnants with the same labelling, was studied in the two groups of rats. Ten minutes after i.v. injection of chylomicron remnants $10.6 \pm 0.5\%$ (means $\pm$ SEM, $n=8$) of the injected [^3H]cholesterol and $7.6 \pm 0.4\%$ of the [^{14}C]linoleic acid was found per g liver in the porta-caval shunted rats while the corresponding figures in the sham operated group ($n=8$) were 6.4 ± 0.4 and 4.9 ± 0.3 respectively ($p < 0.001$ for both ^3H and ^{14}C). Thus despite a $> 40\%$ reduction of liver weight induced by the shunting procedure, the total liver uptake of chylomicron remnants was not significantly decreased. The uptake of chylomicron lipids per unit liver weight was found to be normal in the atrophic livers of porta-caval shunted rats also when very large loads of chyle were administered.

INTRODUCTION

Porta-caval anastomosis (PCA) has been used to lower plasma cholesterol in humans suffering from familial hypercholesterolemia (for refs. see ref. 1). The mechanism underlying this effect is, however, not clear. A decreased plasma cholesterol concentration following PCA has been reported in monkeys (2), dogs (3), swine (4) and rats (5-11). The magnitude of the cholesterol-lowering effect of PCA obtained in different studies in the rat varies considerably, the variations being at least partly depending on different dietary regimens. One prominent effect of the PCA is a decrease in the plasma $d < 1.006$ lipoprotein cholesterol (11) or protein (6,7) content, possibly due to a diminished very low density lipoprotein (VLDL) synthesis (6). Evidence for a reduced synthesis of low density lipoprotein (LDL) and high density lipoproteins (HDL) has also been obtained in swine (4). The intestine is, however, likely to produce large amounts of chylomicrons in response to a fat load also in the PCA animal. Yet, studies of the metabolism of chylomicrons in PCA animals are lacking. Since chylomicron remnants are normally cleared very rapidly by the liver (12,13), probably by an endocytic process (14) involving the apoE-receptor (15,16), the circulatory changes and the ensuing liver atrophy in the PCA animals might be expected to delay the remnant clearance. This would lead to an accumulation of apoE- and cholesteryl-ester-rich lipoproteins in plasma, and would also interfere with the metabolic transformation in the liver of dietary fat-soluble vitamins.

The flow of remnant cholesterol to the liver is also an important regulator of hepatic cholesterol synthesis in the rat (17,18). The reports on hepatic cholesterol metabolism after PCA are diverging, e.g. the activity of hepatic hydroxymethylglutaryl-CoA reductase has been reported to be both decreased (7,8) and increased (19) in PCA rats. Also the estimations of choleste-

rol catabolism are conflicting, suggesting either decreased (11,19,20), unchanged (21) or increased (22) bile acid synthesis after PCA.

We have earlier studied the hepatic uptake of chylomicron remnants in cholestatic rats and found it to be severely inhibited (13), probably due to the increased plasma concentration of bile acids. The PCA rat thus provides another interesting test model, having a moderately increased bile acid concentration in blood but completely lacking the hyperlipidemia found in the cholestatic rats.

In the present study we examined the metabolism of chylomicron triacylglycerol and chylomicron remnants in PCA rats. The main purpose was to see, whether there is a delay in the clearance of chylomicron remnants in PCA rats, or whether these particles are metabolized efficiently as is HDL and LDL in swine with PCA (4).

METHODS

Animals

Male Wistar rats were used for the experiments. Using diethyl-ether anesthesia, rats weighing 230-270 g were subjected to either an end-to-side porta-caval shunt (23,24) or a sham operation, which except for the shunt had the same extent and duration as did the shunting procedure. All animals recovered rapidly after the operations and showed no apparent sign of abnormal behaviour. Throughout the study all animals had free access to a standard laboratory diet.

Lipoproteins

250 μCi [$1\alpha, 2\alpha\text{-}^3\text{H}$]cholesterol, 125 μCi [$1\text{-}^{14}\text{C}$]linoleic acid (Radiochemical Centre, Amersham, Bucks, U.K.) and 20 mg cholesterol in 0.5-0.75 ml corn oil were administered intragastrically,

divided in 3-4 doses during a 6 hrs period, to rats with a thoracic duct fistula (25). Radioactively labelled chyle was collected on ice during 12-16 hrs. The chyle was kept at +4°C in the presence of 2 mM Na₂EDTA and used within 1 week. Chylomicron remnants were prepared by injecting radioactively labelled chylomicrons, isolated as described in (26) and containing 3-4 mg cholesterol, into functionally hepatectomized rats (12) which were infused with ≈ 1 ml/h 5% glucose 0.45% NaCl through a jugular vein catheter. After 2 hrs the animals were again anesthetized with diethylether and killed by aortic bleeding. Na₂EDTA (≈ 1 mg/ml) was added to the blood, plasma was prepared and adjusted to $d=1.063$ using a stock solution of NaCl/KBr ($d=1.35$), before layering under saline ($d=1.006$). Ultracentrifugation was performed at 10°C in a MSE 6x14 ml swing-out rotor at 110,000xg for 16 hrs and repeated once under the same conditions. Chylomicron remnants were stored at +4°C and used within 36 hrs.

Experimental design

Three types of experiments were performed using either native chyle or chylomicron remnants. In all cases the lipoproteins were injected into the left jugular vein during light diethylether anesthesia. The rats were allowed to wake up and then again at the indicated times anesthetized and killed by aortic bleeding. Blood was allowed to clot at room temperature and serum separated.

In one series of experiments the animals were injected with 300 μ l preformed chylomicron remnants containing 77 μ g cholesterol, 182 μ g triacylglycerols ($\approx 15\%$ of the initial chylomicron triacylglycerol content), 158×10^3 cpm [³H]cholesteryl esters, 17×10^3 cpm free [³H]cholesterol and 21×10^3 cpm in [¹⁴C]lipids of which 79% was [¹⁴C]triacylglycerols. In other experiments native chyle in two different doses was used. In "low dose" experiments 250 μ l chyle containing 108 μ g cholesterol, 3.0 mg triacylglycerols

rols, 220×10^3 cpm [^3H]cholesteryl esters, 93×10^3 cpm free [^3H]cholesterol, 635×10^3 cpm [^{14}C]lipids of which 89% was [^{14}C]triacylglycerols were injected. In "high dose" experiments 1 ml chyle containing 645 μg cholesterol, 40 mg triacylglycerols, 2.12×10^6 cpm [^3H]cholesteryl esters, 1.43×10^6 cpm free [^3H]cholesterol, 4.30×10^6 cpm [^{14}C]lipids, of which 88% was [^{14}C]triacylglycerols, was injected.

Analytical

Lipids were extracted from aliquots of serum and liver and subjected to thin layer chromatography, and radioactivity was determined as described earlier (13). Cholesterol and triacylglycerols were determined using enzymatic methods (Boehringer Mannheim GmbH, West Germany). Phosphorus content in the lipid extracts was determined according to Brante (27). Bile acids were determined using a fluorometric method (Nyegaard & Co. A/S, Oslo, Norway). Commercial radioimmunoassay kits were used for quantification of estradiol (Biodata, Hypolab S.A., Coisins, Switzerland or Sorin Biomedica, Saluggia, Italy) and testosterone (Sorin Biomedica). Lipoprotein lipase activity was determined against a [^3H]triolein substrate (28). One mU was defined as the enzyme activity able to liberate 1 nmol fatty acids per minute. The activity was determined in reconstituted acetone:ether extracted tissue samples and correlated to protein content (29).

Values are presented as means $\pm$ S.E.M. Data were statistically evaluated using the Student's t-test.

RESULTS

Body weight and liver weight

The body weight of the PCA rats declined immediately postoperatively. Two to three weeks after surgery, i.e. at the time of experiment, the body weight in the PCA group was stable or

slightly increasing. To compensate for the $\approx 20\%$ weight gain by the sham operated animals during this period, PCA rats of a higher initial weight were chosen. Thus at the time when the experiments were performed, the body weight was equal in the two groups (PCA 276 ± 9 g, sham 275 ± 7 g). In spite of this the mean liver weight in the PCA rats (6.2 ± 0.2 g) was only 53% of that in the sham operated group (11.6 ± 0.4 g, $p < 0.001$).

Serum lipid concentrations

The serum triacylglycerol concentration in the PCA group was reduced to 45% of that in the sham operated group. Also the serum phospholipids were decreased by the PCA. No significant difference in total serum cholesterol was seen between the two groups in this study. Serum bile acids and estradiol were increased and testosterone decreased in the PCA group (Table 1).

Liver uptake of chylomicron remnants

Ten minutes after the injection of radioactively labelled chylomicron remnants, equal amounts of radioactive lipids were found in the livers of both PCA and sham operated rats (Table 2). The uptake of remnants per unit liver weight was thus increased in the PCA livers to a degree sufficient to compensate for the reduced liver mass.

Also after injection of native chyle the PCA animals incorporated a greater fraction of both [^3H]cholesterol and [^{14}C]linoleic acid per gram liver than did the sham operated rats. This was observed also when the animals received a very large load of chyle, roughly corresponding to the amount produced during 2-3 hrs in a fat-fed rat (Table 2). In this case, however, the increase was not great enough to compensate for the reduced liver mass in the PCA animals, and thus the total liver uptake of this unphysiologically large dose of lipids was decreased in the PCA animals.

Intravascular metabolism of chylomicrons

High doses of chyle [^{14}C]triacylglycerols were cleared somewhat faster from serum in the PCA than in the sham operated group (Table 3). The increased extrahepatic removal of chyle [^{14}C]triacylglycerols in the PCA animals (Table 4) occurred in spite of a decreased specific activity of lipoprotein lipase in epididymal fat and an unchanged activity in muscle tissue (Table 5).

In contrast to the findings after chyle injection, significantly more [^{14}C]triacylglycerols was found in serum of PCA rats 10 min after the injection of chylomicron remnants, which had already lost 85% of their original triacylglycerol content (Table 3). The serum recovery in the PCA group might, however, be somewhat exaggerated in comparison with the sham operated group since the total serum volume was calculated as 4 ml per 100 g body weight in both groups (30).

The ratio of [^{14}C]triacylglycerols to [^3H]cholesteryl esters in serum might be taken as a rough estimate of size of the chylomicron remnants remaining in the circulation at the end of the experiment. After chyle injection this ratio was lower in the PCA than in the sham operated group, indicating that the triacylglycerol hydrolysis was more extensive and the remnant particles formed thus smaller. This would possibly favour their rapid uptake in the PCA livers (31). It is, however, not likely to be the only explanation for the increased uptake of chyle lipids per gram liver in the PCA animals, since the most significant difference in this respect was seen after injection of chylomicron remnants. In this case the [^{14}C]triacylglycerol to [^3H]cholesteryl ester ratio was actually lowered more in the sham operated than in the PCA rats (Table 3). The absolute recoveries of both these lipids in serum were, however, very low and the [^3H]chole-

steryl ester pool might receive different contributions by esterification of [^3H]cholesterol via the lecithin:cholesterol acyltransferase (LCAT) mechanism in the two groups of animals.

Extrahepatic uptake

As shown in Table 4, the recovery of both total [^3H]cholesterol and [^{14}C]lipids in serum and liver together did not differ significantly between PCA and sham operated rats after injection of preformed chylomicron remnants. However, after injection of larger amounts of lipids as whole chyle, the PCA animals seemed to metabolize somewhat more of both types of lipids extrahepatically than did the sham operated animals (Table 4). Although the uptake of total [^3H]cholesterol in heart, lungs, kidneys and spleen was higher in the PCA than in the sham operated animals (Table 6), the uptake in these organs was very low (1.5%), in comparison with the uptake in the liver (>90%). The uptake of [^3H]cholesterol in adrenals was, in contrast to all other organs studied, significantly lower in the PCA than in the sham operated group.

DISCUSSION

One important step in the conversion of chylomicrons to chylomicron remnants is the intravascular hydrolysis of most of the triacylglycerols by the action of lipoprotein lipase (12). This enzyme is known to be reduced in adipose tissue from starved rats (32) and the same finding would thus be expected in PCA rats, which could be characterized to be in a chronically underfed state (24,30). Indeed the lipoprotein lipase activity was found to be decreased in epididymal fat but unchanged in muscle (Table 5). In spite of this the clearance of [^{14}C]triacylglycerols, even after injection of very large doses of chyle, was found to be more rapid in PCA rats than in sham operated ones (Table 3,4). The reason for this difference is not clear. Al-

though the PCA animals have a significantly reduced level of serum triacylglycerols (Table 1, Refs. 5,6,10,11) this would not be expected to affect the rate of chylomicron triacylglycerol hydrolysis, since the amount of chyle triacylglycerols injected in the "high dose" experiments in this study was about five-fold higher than the endogenous triacylglycerol pool. Furthermore, lipoprotein lipase is known to have a higher catalytic rate with chylomicrons than with VLDL as substrate (33).

The rapid lipolysis of chylomicron triacylglycerols was also reflected in a lower [^{14}C]triacylglycerol/[^3H]cholesteryl ester ratio in the remnants remaining in plasma in the PCA animals (Table 3). Since small size is one factor that is likely to enhance the uptake of remnants by the liver (31) the efficient lipolysis might promote the liver uptake of chylomicron [^3H]cholesteryl ester in the PCA rats. However, the increased uptake of chylomicron remnants per gram liver in the PCA animals was most clearly demonstrated when compared with the sham operated group after injection of preformed chylomicron remnants, in which already 85% of the initial triacylglycerols had been hydrolyzed (Table 2). In these experiments the fraction of injected remnant triacylglycerols retained in serum was higher in the PCA than in the sham operated animals (Table 3).

The high uptake of chylomicron remnants in the PCA liver may not be immediately explained by circulatory changes (34,35). Several other mechanisms might be suggested to influence the remnant clearance in the PCA rat. First, the distribution of apoproteins in plasma, which has not been studied in PCA animals, might be different in the two groups of rats. However, if the increased remnant clearance seen in the PCA rats would be explained by such differences, a very rapid change in the apopro-

tein composition must be assumed in the preformed chylomicron remnants, which were eliminated faster in the PCA than in the normal rats, in spite of their common origin.

Second, the mechanism for hepatic uptake of chylomicron remnants might be more directly influenced by the PCA. E.g. Quarfordt et al. have reported an increased hepatic uptake of triacylglycerol-rich lipoproteins during short-time perfusions of isolated livers from fasted rats (36). In our study the rats were fed ad libitum and the PCA group was likely to have a lower food intake than the controls. Since Mahley et al. (15) demonstrated increased levels of apoB,E-receptor, i.e. the lipoprotein receptor that binds both apoB- and apoE-containing lipoproteins, in liver membranes from fasted dogs, the differences in nutritional state might cause a difference in the receptor-mediated hepatic lipoprotein uptake between the two groups. In addition, the apoB,E-receptor activity, which may be increased very markedly by treating male rats with ethinylestradiol (37), might also be influenced by the increased serum estradiol/testosterone ratio that was observed in the PCA rats (Table 1).

Most of the chylomicron remnant removal occurs, however, via the apoE- rather than the apoB,E-receptor and is not subject to the same metabolic regulation as the hepatic LDL-removal (38). Although no sign of an increased catabolic rate of LDL has been reported neither in PCA swine (4) nor rats (6), no direct measurements of hepatic uptake has been performed in these studies. We have been unable to demonstrate any increase in chylomicron remnant binding to liver membranes from PCA rats, whereas binding of human ^{125}I -LDL was actually higher than in controls, although far less than in membranes from ethinylestradiol-treated rats (B. Landin, Å. Nilsson, unpublished observa-

tion). Evidence that receptor induction is a major factor for the rapid clearance of chylomicron remnants in the PCA rat is thus lacking.

Third, although chylomicron remnants are normally taken up mainly by hepatocytes (39,40), it is possible that alternate pathways like e.g. the clearance of acetoacetylated LDL by reticuloendothelial cells (41), might play a relatively prominent role in lipoprotein clearance in PCA rats. The uptake of chylomicron remnants in spleen and lungs was actually higher in the PCA group (Table 5), suggesting that such mechanisms might be more effective in these animals. However, the hepatic RES function, determined by carbon clearance, has been shown to be markedly decreased after PCA (42). Furthermore, the radioactivity per gram tissue was severalfold higher in the liver than in spleen and lungs (Table 2,6). There was thus no evidence for a major change in the pathways by which chylomicron remnants are catabolized in the two groups of animals, although nonparenchymal liver cells might contribute to the increased hepatic uptake of chylomicron remnants in PCA rats.

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Table 1. Serum lipids in PCA and sham operated rats

	n	PCA	n	sham
Total cholesterol (mg/ml)	23	0.44 $\pm$ 0.01	18	0.46 $\pm$ 0.02
Triacylglycerols (mg/ml)	15	0.78 $\pm$ 0.13**	14	1.73 $\pm$ 0.28
Phospholipids (mg/ml)	18	1.71 $\pm$ 0.03***	15	2.04 $\pm$ 0.04
Bile acids (nmol/ml)	13	133 $\pm$ 7***	9	15 $\pm$ 3
Estradiol (pg/ml)	17	17 $\pm$ 3*	17	9.1 $\pm$ 1
Testosterone (ng/ml)	6	2.8 $\pm$ 0.6*	6	8.0 $\pm$ 2

* p < 0.05

** p < 0.01

*** p < 0.001

Table 2. Liver lipid radioactivity after injection of labelled chyle in different doses or chylomicron remnants

	n	Time (min)	Total [³ H]cholesterol recovered		[¹⁴ C]lipids recovered		CE ^a C
			% per g	% per liver	% per g	% per liver	
<u>Remnants</u>							
PCA	8	10	11 ± 0.5***	91 ± 4	7.6 ± 0.4***	65 ± 5	0.25 ± 0.01
sham	8	10	6.4 ± 0.4	95 ± 5	4.9 ± 0.3	73 ± 4	0.24 ± 0.01
<u>Chyle</u>							
Low dose							
PCA	2	15	8.1	54	3.2	22	0.64
sham	2	15	5.7	64	2.5	28	0.52
PCA	2	30	11	66	3.0	18	0.40
sham	2	30	8.1	82	2.0	20	0.20
High dose							
PCA	3	15	1.8 ± 0.2	14 ± 1*	1.0 ± 0.1*	7.8 ± 0.6	0.55 ± 0.03
sham	3	15	1.5 ± 0.1	20 ± 2	0.7 ± 0.05	9.0 ± 0.7	0.63 ± 0.03
PCA	3	30	4.4 ± 0.9	23 ± 3*	1.9 ± 0.2*	11 ± 1*	0.59 ± 0.02
sham	3	30	2.9 ± 0.1	40 ± 2	1.1 ± 0.05	15 ± 1	0.60 ± 0.02

^aRatio between cpm [^3H]cholesteryl ester and [^3H]cholesterol in liver, divided with the corresponding ratio in the injected lipoprotein.

* $p < 0.05$ *** $p < 0.001$

Table 3. Serum lipid radioactivity after injection of labelled lipoproteins^a

	n	Time (min)	Total [³ H] cholesterol recovered % in serum ^b	[¹⁴ C] triacylglycerols recovered % in serum	$\frac{[^{14}\text{C}] \text{ TAGC}}{[^3\text{H}] \text{ CE}}$
<u>Remnants</u>					
PCA	8	10	13 ± 3 ^{**}	9.2 ± 2 ^{**}	0.72 ± 0.01 ^{***}
sham	8	10	3.6 ± 0.8	1.5 ± 0.5	0.32 ± 0.07
<u>Chyle</u>					
<u>Low dose</u>					
PCA	2	15	41	9.3	0.11
sham	2	15	41	21	0.39
PCA	2	30	23	6.7	0.24
sham	2	30	12	6.8	0.48
<u>High dose</u>					
PCA	3	15	63 ± 4	43 ± 5	0.40 ± 0.04 [*]
sham	3	15	75 ± 4	62 ± 5	0.58 ± 0.02
PCA	3	30	39 ± 4	14 ± 2	0.19 ± 0.04 [*]
sham	3	30	35 ± 3	22 ± 3	0.40 ± 0.02

^aData from the same experiments as in Table 2.^bCalculated as 4 ml/100 g body weight.^cRatio between cpm [¹⁴C]triacylglycerols and [³H]cholesteryl esters in serum, divided with the corresponding ratio in the injected lipoprotein^{*} p < 0.05^{**} p < 0.01^{***} p < 0.001

Table 4. Recovery of [^3H]cholesterol^a and [^{14}C]lipids in serum and liver

	n	Time (min)	Total recovery	
			[^3H]cholesterol	[^{14}C]lipids
			(%)	(%)
<u>Remnants</u>				
PCA	8	10	104 $\pm$ 5	75 $\pm$ 5
sham	8	10	98 $\pm$ 4	74 $\pm$ 4
<u>Chyle</u>				
Low dose				
PCA	2	15	95	26
sham	2	15	105	33
PCA	2	30	88	14
sham	2	30	94	13
High dose				
PCA	3	15	77 $\pm$ 4*	45 $\pm$ 6
sham	3	15	95 $\pm$ 3	68 $\pm$ 4
PCA	3	30	62 $\pm$ 5	21 $\pm$ 2*
sham	3	30	75 $\pm$ 1	32 $\pm$ 3

^afree + esterified

^bdata from the same experiments as in Table 2 and 3

* $p < 0.05$

Table 5. Lipoprotein lipase activity in epididymal fat and thigh muscle

	n	Lipoprotein lipase activity (mU/mg protein)	
		Fat	Muscle
PCA	6	2.2 $\pm$ 0.6*	0.37 $\pm$ 0.09
sham	6	4.2 $\pm$ 0.6	0.34 $\pm$ 0.08

*
p < 0.05

Table 6. Uptake of chylomicron remnant [^3H]cholesterol^a in different tissues^b

		Recovery (% of injected)	
		per g	per organ
Heart	PCA	<0.06	<0.06
	sham	<0.02	<0.02
Lungs	PCA	0.18 $\pm$ 0.03**	0.23 $\pm$ 0.03*
	sham	0.07 $\pm$ 0.01	0.13 $\pm$ 0.02
Kidneys	PCA	0.05 $\pm$ 0.01*	0.12 $\pm$ 0.02*
	sham	0.02 $\pm$ 0.004	0.05 $\pm$ 0.01
Adrenals	PCA	0.61 $\pm$ 0.10	0.03 $\pm$ 0.01*
	sham	0.87 $\pm$ 0.32	0.06 $\pm$ 0.01
Spleen	PCA	1.34 $\pm$ 0.07***	1.00 $\pm$ 0.07***
	sham	0.36 $\pm$ 0.06	0.29 $\pm$ 0.04
Testes	PCA	<0.01	<0.01
	sham	<0.01	<0.01
Fat	PCA	<0.03	-
	sham	<0.02	-
Muscle	PCA	<0.01	-
	sham	<0.01	-

^aFree + esterified

^bValues obtained 10 min after injection of labelled chylomicron remnants, see also Table 2 and 3, n=8 in both groups.

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Inhibition of Hepatic Chylomicron Remnant Uptake in the Cholestatic Rat

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[³H]retinol- or [³H]cholesterol- and [¹⁴C]fatty acid-labeled chylomicrons and chylomicron remnants were injected intravenously into normal rats and rats subjected to partial or total obstruction of the bile duct 8 or 48 h earlier. The clearance from plasma of the [³H]retinyl and the [³H]cholesteryl esters in 30–120 min was markedly decreased in rats with total obstruction, and the uptake of ³H by the liver was significantly less than in control rats. The elimination of the [¹⁴C]triacylglycerol of the native chyle lipoprotein was not significantly affected, and the delayed plasma clearance was seen also when the [³H]lipid ester radioactivity was injected as chylomicron remnants prepared in vitro. If only one of the two main bile duct branches was ligated, the uptake of radioactivity did not differ markedly in the obstructed and unobstructed part of the liver, and only small effects on the plasma disappearance were seen. The defective clearance of the chylomicron remnants was thus not related to the obstructed bile flow per se but rather to the consequences of the total obstruction, such as the high plasma bile acid level or the accumulation of abnormal lipoproteins in plasma.

Key-words: Bile acids; cholestasis; cholesteryl ester; chylomicron; chylomicron remnant; retinyl ester

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An abnormal plasma lipoprotein composition in the cholestatic situation is a well-known phenomenon in several species (5, 8, 18). In the rat, as in humans, a significant rise in phospholipids and cholesterol occurs, and abnormal lipoprotein particles like lipoprotein X can be demonstrated (8, 12, 18). Moreover, in patients with obstructive jaundice (16, 24), and recently also in cholestatic rats (21), another pathological low-density lipoprotein (LDL) fraction rich in triacylglycerols has been described. Although the perfused rat liver secretes lipoprotein X (12), the origin of the abnormal phospholipid-rich particles has not been completely clarified. For instance, lipoprotein X is also found in blood when large amounts of phospholipid-triacylglycerol emulsion (Intralipid) is infused intravenously (7). It is thus possible that a defective clearance of lipoprotein unesterified cholesterol and phospholipids also con-

tributes to the hyperlipidemia in cholestasis. It has also been suggested that the accumulation of bile acids or other bile components, rather than the cholestasis per se, changes the lipoprotein metabolism, since animals whose bile duct had been anastomosed to the vena cava also developed severe hypercholesterolemia and hyperphospholipidemia (17).

The interruption of the bile flow leads to a decreased fat absorption, but in the presence of a normal pancreatic function a significant digestion and absorption of triacylglycerol, and thereby a significant chylomicron secretion, still takes place (28). In the present study we examined the metabolism of chylomicron triacylglycerol and of the chylomicron remnant constituents (29) cholesteryl and retinyl ester in rats with complete or partial biliary obstruction and in normal controls. The study was undertaken for four reasons: First,

the severe changes in the plasma lipoproteins in cholestasis might interfere with the formation of normal chylomicron remnant particles. If so, studies of the cholestatic situation might give clues as to which of the changes occurring during the conversion of chylomicrons to remnants (14, 22) are necessary for the avid uptake of normal remnants by the liver (9, 29, 31). Second, a defective removal of remnant particles from blood would be of interest in relation to whether the abnormal triacylglycerol-rich LDL of cholestatic plasma (16, 21, 24) may be formed as a remnant from very low density lipoproteins (VLDL) or chylomicrons. Third, a defective lipoprotein uptake by the liver and thereby a defective feedback control (26) might explain the increase in hepatic cholesterol synthesis that occurs after bile duct ligation (11, 15, 32). Fourth, it would prevent the metabolic transformation in the liver and thereby the biological action of any fat-soluble vitamins transported by the lymph in cholestasis.

MATERIALS AND METHODS

Material. Male white Sprague-Dawley rats (250–300 g) were purchased from Anticimex AB, Stockholm, Sweden; [^3H]retinol (all-trans) from New England Nuclear, Dreieich, West Germany; [^{14}C]linoleic acid, [^{14}C]palmitic acid, and [^3H]cholesterol from the Radiochemical Centre (Amersham, Bucks, England); retinol and retinyl palmitate from Sigma Chemical Co. (St. Louis, Mo., USA); and 13-cis retinyl acetate from Ferrosan AB, Malmö, Sweden. Sterognost-3a® Flu was obtained from Nyegaard & Co. A/S, Oslo, Norway.

Methods. Thoracic duct cannulations were performed according to Bollman et al. (6). Details have been given earlier (13). Labeled chyle was obtained by feeding 0.5 ml of corn oil mixed with either 250 μCi [^3H]retinol (all-trans), 1 mg unlabeled retinol, 125 μCi [^{14}C]linoleic acid, and 20 mg cholesterol, or 250 μCi [^3H]cholesterol and 125 μCi [^{14}C]palmitic acid. Chylomicrons ($S_r > 400$) were prepared according to Minari & Zilversmit (20). Chylomicron remnants were prepared by incubating chyle with post-heparin rat plasma as described by Nilsson (27).

To examine whether the [^3H]retinyl ester remaining in plasma after 30–120 min was still in macromolecular aggregates with the size of remnants, whole serum or total lipoproteins, prepared by ultracentrifugation at $d = 1.225$ for 24 h at 200,000 g, were subjected to gel chromatography on Biogel A-15 (30).

Non-fasted rats were anesthetized with diethyl-ether, and the common bile duct was doubly ligated, carefully avoiding interference with the pancreatic tissue. To produce obstruction of either the left (major) or the right (minor) part of the liver, only one of the two bile duct branches was ligated. In some series of experiments the bile duct was divided between ligatures. The rats had free access to water and ordinary laboratory chow after the operation.

Before the experiments the rats were again anesthetized with diethylether, and chyle, chylomicrons, or chylomicron remnants (suspended in saline) were injected intravenously into the jugular vein. The animals were then allowed to wake up and after various times they were again anesthetized and killed by aortic bleeding. Blood was collected in cooled tubes containing ≈ 1 mg disodium-EDTA/ml. Plasma was separated and analyzed for radioactivity of different lipid classes and, in some cases, for cholesterol and phospholipid mass. The liver was perfused with 20 ml ice-cold saline through the inferior vena cava before extirpation.

Analytical. Aliquots of lipoproteins, plasma, and liver were extracted with chloroform/methanol, 1:1. In cases of [^3H]retinyl ester-labeling the organic solvents contained 0.05% butylated hydroxytoluene, and 50 μl of a solution containing 10 mg retinyl ester and 10 mg retinol/ml was added to each sample. All samples containing retinyl ester-label were handled in dim light. After removal of the precipitate, proportions were adjusted to 2:1:1 (chloroform/methanol/water). The resulting upper phase was discarded and the lower chloroform phase concentrated under nitrogen. Aliquots of the lipid extract were subjected to thin-layer chromatography on silica gel G. The mobile phase was light petroleum/diethylether/acetic acid, 80:20:1 (v/v). Retinyl ester and retinol were visualized

with ultraviolet fluorescence and other lipids with iodine vapor. Spots were scraped into counting vials, and radioactivity was determined in a Packard Tricarb Liquid Scintillator (model 3375) in 10 ml toluene-based scintillation fluid to which 1 ml of methanol was added. Quenching was corrected for by using an external standard.

Retinol content in the chyle was determined by high-performance liquid chromatography on a Waters model ALC/GPC-204 liquid chromatograph after alkaline hydrolysis of the retinyl esters. The column was packed with Nucleosil C₁₈ and the mobile phase was water/methanol 25:75 (v/v). 13-cis retinol was used as internal standard. Cholesterol was determined according to Zak et al. (33) after saponification of the lipid extract (1). Triacylglycerols were determined by gas-liquid chromatography of the fatty acid methyl esters (2). Lipid phosphorus was determined according to Bartlett (3) as modified by Morrison (23). Plasma bile acid concentration was determined using the Sterognost-3 α Flu test.

The results were statistically evaluated using Student's *t* test. The confidence intervals given in the tables are * = *p* < 0.05, ** = *p* < 0.01, *** = *p* < 0.001.

RESULTS

Eight hours after ligation of the bile duct the plasma concentration of both cholesterol and phospholipids increased twofold. The bile acid concentration in plasma rose drastically from the very low normal level to values above 1 mM. Forty-eight hours after ligation the cholesterol and phospholipid increase in plasma was more pronounced, whereas the bile acid concentration was about the same as after 8 h. The plasma triacylglycerol concentration was only slightly elevated. Obstruction for 48 h of the bile duct draining the left liver lobes, comprising 70%–80% of the liver mass ('major obstruction'), caused much smaller changes in the plasma lipid and bile acid levels than the total bile duct obstruction. Ligation of the bile duct system draining the right liver lobe ('minor obstruction') did not produce any changes except a slight increase in the plasma cholesterol level (Table I).

Table I. Plasma lipids in cholestatic rats: plasma lipid concentration in rats with various degrees of bile duct obstruction compared with normals

Group	N	Cholesteryl ester (μg/ml)	Cholesterol (μg/ml)	Lipid phosphorous (μg/ml)	Triacylglycerol (μg/ml)	Bile acids (nmol/ml)
No obstruction	9	513 ± 21	192 ± 14	59 ± 1.9	407 ± 30	9.8 ± 3.6
Total obstruction 8 h	3	572 ± 78	450 ± 23***	94 ± 2.5***	601 ± 181	1425 ± 405***
Total obstruction 48 h	9	667 ± 51*	1934 ± 514**	216 ± 38***	559 ± 34**	1331 ± 211***
Major obstruction 48 h	7	804 ± 75*	425 ± 32***	87 ± 4.9***	486 ± 21	58 ± 12***
Minor obstruction 48 h	3	582 ± 56	288 ± 4.4**	60 ± 0.7	441 ± 179	11 ± 1.5

N = number of rats. Values are means ± S.E.M. For further details on the different types of ligation see Results. Significant changes are indicated as described in Methods. When not otherwise stated, the differences are not significant.

When [^3H]retinyl ester-labeled chyle was injected intravenously into normal or sham-operated rats, about 95% of the radioactivity was eliminated from plasma in 30 min. After this time, the plasma radioactivity increased slightly, but this was caused by a continuous increase in the fraction of unesterified retinol, probably reflecting the release from the liver of retinol bound to retinol-binding protein (RBP) (25). In cholestatic rats, however, the plasma elimination of ^3H -radioactivity was significantly delayed; e.g., more than 50% still remained in plasma after 30 min. As the total plasma volume was assumed to be 4 ml/100 g body weight in the calculations, any change in the ratio between weight and plasma volume induced by the cholestatic state might influence these values. However, changes of the magnitude seen here could not be explained in this way. Furthermore, the ratio of esterified to unesterified retinol did not decrease at a normal rate. Two hours after injection as much as 70% of the plasma ^3H -radioactivity was still found as retinyl ester (Table II).

In rats with only one of the major branches of the bile duct ligated, the proportion of the injected [^3H]retinyl ester that remained in plasma after 60 min was also larger than in the controls. This difference was larger in the rats with 'major' than those with 'minor' obstruction (Tables I and II). The inhibition of the remnant clearance was, however, far less than in rats with complete obstruction.

In contrast to the [^3H]retinyl ester the chylomicron [^{14}C]triacylglycerol was eliminated from plasma in an almost normal manner in the cholestatic rats (Table II). That the defective retinyl ester clearance was not caused by a slow hydrolysis of chylomicron triacylglycerol by lipoprotein lipase in the cholestatic rats was also indicated by the finding that the [^3H]retinyl ester of chylomicron remnants prepared *in vitro* was cleared as poorly as that of [^3H]retinyl ester of injected native chylomicrons (Table II).

The [^3H]retinyl ester remaining in plasma 30–120 min after injection of chylomicrons was mainly in large lipoproteins migrating with the void volume on a Biogel A-15 column. However, a certain amount of ^3H radioactivity was also

found to be associated with a spectrum of somewhat smaller particles included in the gel (Fig. 1). In some cases a radioactivity peak was found, roughly corresponding to the elution volume expected for LDL, as calculated from the experiments of Rudel *et al.* (30).

When [^3H]retinyl ester-labeled serum from cholestatic rats injected with chylomicrons was injected intravenously into normal rats, the clearance of [^3H]retinyl ester was essentially complete in 30 min. The labeled particles were thus efficiently cleared, like normal remnant particles, in normal recipient animals.

The liver uptake of ^3H radioactivity was also markedly decreased in the rats with complete obstruction (Table II). The ratio of [^3H]retinyl ester to [^3H]retinol radioactivity in the liver was not changed by cholestasis. Of the liver ^3H radioactivity 60%–80% was found as retinyl ester in all groups of rats and at all times studied. In rats with partial obstruction the uptake did not differ significantly between the obstructed and the unobstructed lobes, and the uptake per liver weight was close to that in the control rats (Table II).

Since the liver ^3H radioactivity encountered in the experiments described above does not show whether the decreased liver uptake was also associated with a decreased hydrolysis of the chylomicron remnant cholesteryl ester, all experiments on rats with different degrees of bile duct obstruction were also performed using [^3H]cholesterol-labeled chyle. The findings with regard to decreased plasma elimination and delayed liver uptake, as described for [^3H]retinyl ester-labeled chyle, were confirmed (Table III). In the rats with partial obstruction the ratio of unesterified to esterified [^3H]cholesterol increased with time, as in the normal controls, indicating that the hydrolysis of [^3H]cholesteryl ester after the uptake proceeded normally. In rats with complete obstruction the ratio was also normal, despite the slow remnant uptake (Table III).

DISCUSSION

The present study demonstrates that the clearance from blood of the cholesteryl and the retinyl

Table II. Plasma and liver radioactivity after injection of [³H]retinyl ester [¹⁴C]triacylglycerol-labeled chyle or chylomicron remnants into normal and cholestatic rats

Group	Time (min)	N	Plasma ³ H recovery (%)	% of plasma ³ H as retinyl ester (%)	Plasma [¹⁴ C]TAG-recovery (%)	Liver ³ H recovery (%)	
						Nonobstructed	Obstructed
Chyle							
No obstruction	10	9	34.3 ± 3.3	93.5 ± 1.7	21.2 ± 4.9	43.5 ± 5.1	—
	30	3	4.1 ± 0.4	64.7 ± 8.8	2.3 ± 0.6	69.5 ± 11.2	—
	60	4	7.8 ± 2.1	11.1 ± 2.3	4.6 ± 3.6	45.3 ± 6.0	—
	120	4	10.7 ± 1.6	4.1 ± 0.4	0.4 ± 0.2	38.9 ± 11.8	—
Total obstruction (8 h)	10	3	73.5 ± 11.7***	93.1 ± 1.6	6.3 ± 0.03	—	15.9 ± 2.6*
	30	3	58.8 ± 0.9***	94.7 ± 0.5*	2.5 ± 0.5	—	60.4 ± 11.9
	60	2	37.8 ± 0.3***	88.7 ± 3.0***	1.6 ± 0.6	—	42.9 ± 3.1
	120	3	28.6 ± 8.0*	67.6 ± 8.9***	0.9 ± 0.2	—	39.1 ± 4.8
Total obstruction (48 h)	10	6	97.4 ± 8.6***	96.6 ± 0.6	37.1 ± 1.7	—	10.7 ± 1.4***
	30	5	81.3 ± 4.0***	96.0 ± 0.3**	16.3 ± 3.4	—	20.5 ± 1.4**
	60	4	50.9 ± 5.8***	90.2 ± 2.3***	4.9 ± 1.3	—	30.4 ± 8.7
	120	3	22.2 ± 6.1	69.5 ± 12.2**	2.1 ± 1.4	—	28.3 ± 11.9
Major obstruction	10	3	59.6 ± 17.2*	95.0 ± 0.2	23.5 ± 0.5	52.6 ± 21.3	36.1 ± 12.6
	60	6	8.6 ± 0.7	33.7 ± 10.2	4.6 ± 1.2	72.5 ± 4.6	66.9 ± 6.7
Minor obstruction	10	2	30.3 ± 12.3	94.6 ± 1.9	6.6 ± 5.6	37.3 ± 9.8	41.9 ± 8.2
	60	6	8.2 ± 0.7	19.8 ± 2.8	1.3 ± 0.1	55.1 ± 4.2	61.2 ± 4.6
Chylomicron remnants							
No obstruction	10	3	13.3 ± 7.3	96.8 ± 1.2	—	66.3 ± 13.6	—
	30	3	3.1 ± 0.9	45.7 ± 1.8	—	75.8 ± 4.7	—
	60	3	5.7 ± 0.4	24.5 ± 11.8	—	58.0 ± 5.7	—
	120	2	16.1 ± 1.0	5.2 ± 0.9	—	53.8 ± 1.2	—
Total obstruction	10	2	60.6 ± 1.1*	95.9 ± 0.4	—	—	18.5 ± 3.2
	30	2	54.2 ± 2.4***	96.5 ± 0.8***	—	—	29.2 ± 1.1**
	60	7	69.2 ± 6.6***	95.0 ± 0.7***	—	—	20.1 ± 3.0***
	120	2	27.4 ± 10.2	86.1 ± 2.2***	—	—	33.4 ± 3.6*

Rats were injected with 0.2–0.3 ml [³H]retinyl ester-labeled chyle or chylomicron remnants. The injected material contained, per ml, $1.67 \pm 0.22 \times 10^6$ cpm ³H ($90.1 \pm 2.0\%$ as retinyl ester and $3.1 \pm 0.3\%$ as retinol) and $1.25 \pm 0.26 \times 10^6$ cpm ¹⁴C ($88.0 \pm 1.0\%$ as triacylglycerol (TAG)), 45.1 ± 5.5 µg retinol, 7.0 ± 1.1 mg triacylglycerol, and 535 ± 45 µg cholesterol. $83.2 \pm 2.0\%$ of the ³H radioactivity was in large chylomicrons ($S_i > 400$). During the preparation of chylomicron remnants with postheparin plasma, 79%–84% of the triacylglycerol was hydrolyzed. In the calculation of the ³H radioactivity and the [¹⁴C]triacylglycerol recovery (as percentage of injected dose) the plasma volume was assumed to be 4 ml/100 g body weight. Values for the liver uptake in non-obstructed and obstructed parts of the liver in rats with partial obstruction have been normalized to uptake per total liver weight to allow direct comparison with the controls. The significant differences marked in the table are those seen when comparing with control rats receiving the same type of lipoproteins.

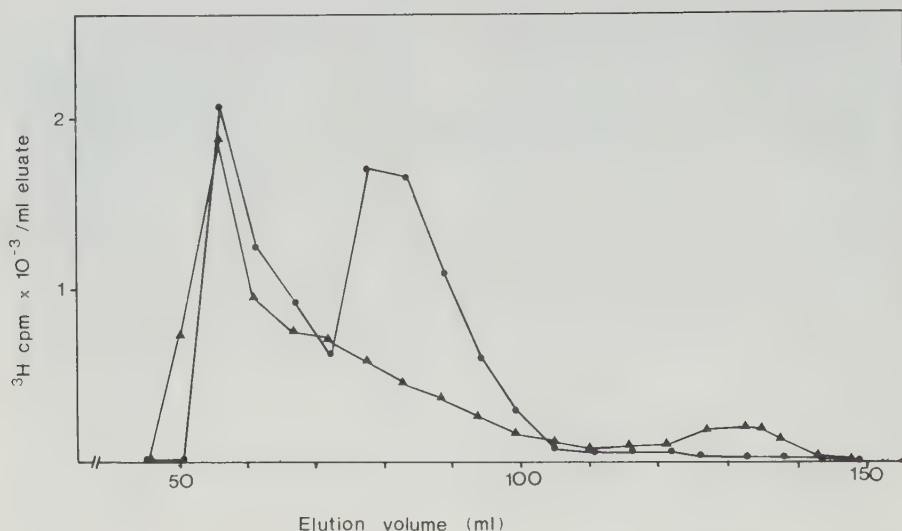


Fig. 1. Gel chromatography of cholestatic serum after injection of [^3H]retinyl ester-labeled chylomicrons. [^3H]retinyl ester-[^{14}C]triacylglycerol-labeled chylomicrons ($S_z > 400$) were injected intravenously into rats subjected to total bile duct obstruction 48 h earlier. Serum bile acid concentrations were 1.5–2.0 $\mu\text{mol/ml}$. In the first experiment (●—●) the chylomicrons injected contained 1.5 mg triacylglycerol and 417,000 cpm ^3H . 30 min later 80% of the injected ^3H radioactivity (92% as retinyl ester) and 23% of the [^{14}C]triacylglycerol (74% of the ^{14}C radioactivity) were found in serum. In the second experiment (▲—▲) 1.0 mg chylomicron triacylglycerol and 292,000 cpm ^3H were given to each rat. 120 min later serum contained 43% of the initial ^3H radioactivity (76% as retinyl ester) and 1.5% of the [^{14}C]triacylglycerol (29% of the ^{14}C radioactivity). Aliquots of serum (≈ 3 ml) were subjected to gel chromatography on a Biogel A-15 column (1.5 $\times$ 90 cm), eluted with 0.15 M NaCl 0.1 mg/ml disodium-EDTA, pH 7.4, at a flow rate of about 7 ml/h. Fractions of about 5 ml were collected, and aliquots of these were subjected to liquid scintillation counting in Instagel.

portions of chylomicrons is markedly delayed in the cholestatic rat. The triacylglycerol clearance was much less affected, indicating that the delayed clearance of the chylomicron remnant constituents was not secondary to a defective lipoprotein lipase action. The [^3H]retinyl ester remaining in plasma was in large particles that contained less than 10% of the [^{14}C]triacylglycerol compared with the injected chylomicrons (Fig. 1). In these respects the particles were thus similar to normal chylomicron remnants and were larger than the abnormal triacylglycerol-rich LDL seen in cholestasis (16, 21, 24). The marked inhibition of the remnant clearance might, however, give an opportunity for intravascular conversion to smaller particles over longer time periods than those studied. Moreover, the injected chylomi-

cons from fat-fed rats are likely to be larger than the average triacylglycerol-rich lipoproteins normally entering blood in the cholestatic rat. More detailed studies including experiments with small chylomicrons are therefore necessary to decide whether there is a direct conversion of chylomicrons or liver VLDL to the abnormal LDL in cholestasis.

The inhibition of the remnant clearance may be due either to a formation of remnant particles with an abnormal composition, which are not avidly cleared by the hepatic mechanism for remnant uptake, or to interference of cholestasis with the hepatic uptake process itself. The findings that normal remnants prepared with postheparin plasma were rapidly cleared in normal but not in cholestatic animals and that [^3H]retinyl ester-

Table III. Plasma and liver radioactivity after injection of [^3H]cholesterol-labeled chyle

Groups	Time (min)	N	Plasma ^3H recovery (%)	Liver ^3H recovery (% of injected dose)		% of liver ^3H as cholesteryl ester	
				Nonobstructed	Obstructed	Nonobstructed	Obstructed
Controls	10	3	27.4 $\pm$ 11.3	30.6 $\pm$ 2.9	—	45.2 $\pm$ 1.4	—
	30	3	8.6 $\pm$ 3.1	63.7 $\pm$ 8.0	—	32.8 $\pm$ 6.1	—
	60	3	7.0 $\pm$ 1.5	61.3 $\pm$ 10.3	—	14.4 $\pm$ 2.1	—
	120	3	8.1 $\pm$ 1.3	41.9 $\pm$ 7.3	—	8.8 $\pm$ 0.9	—
Total obstruction 48 h	10	4	66.8 $\pm$ 5.3*	—	6.7 $\pm$ 0.4***	—	28.3 $\pm$ 3.3**
	30	3	69.2 $\pm$ 8.1**	—	11.8 $\pm$ 2.0**	—	30.2 $\pm$ 3.7
	60	3	43.3 $\pm$ 9.3*	—	31.1 $\pm$ 2.9*	—	25.9 $\pm$ 2.8*
	120	3	21.5 $\pm$ 6.7	—	31.1 $\pm$ 5.0	—	14.9 $\pm$ 3.5
Major obstruction	10	3	59.4 $\pm$ 8.2	35.6 $\pm$ 14.5	29.6 $\pm$ 10.6	35.4 $\pm$ 5.4	36.0 $\pm$ 4.7
	30	3	15.1 $\pm$ 1.6	78.2 $\pm$ 3.4	68.0 $\pm$ 7.9	32.6 $\pm$ 2.3	33.9 $\pm$ 1.6
	60	3	7.5 $\pm$ 2.2	67.4 $\pm$ 11.3	62.9 $\pm$ 10.4	8.7 $\pm$ 1.5	8.6 $\pm$ 0.9
	120	3	13.1 $\pm$ 2.0	47.1 $\pm$ 9.2	46.4 $\pm$ 7.6	6.4 $\pm$ 0.6	7.2 $\pm$ 0.7

The injected chyle (0.2-0.3 ml) contained, per ml, $1.2 \pm 0.2 \times 10^6$ cpm ^3H (53.0 $\pm$ 3.8% as cholesteryl ester), 7.8 $\pm$ 1.5 mg triacylglycerol and 368 $\pm$ 184 μg cholesterol. 76.2% $\pm$ 1.7% of the radioactivity was in large chylomicrons ($S_f > 400$). The recoveries of ^3H radioactivity in estimated plasma volume (4 ml/100 g body weight) and in liver are given as percentage of injected dose. Values for the uptake in different liver lobes in rats with partial obstruction are expressed as described in Table II.

labeled particles of cholestatic serum were rapidly cleared in normal recipient animals speak against the first possibility. The possibility has, however, not been excluded that injected remnants can undergo intravascular changes after injection.

An interference with the cellular uptake of remnants in the liver may be due to the cholestasis per se or to the products accumulating in plasma and liver cells after complete biliary obstruction. To distinguish between these possibilities, the hepatic uptake was studied in rats that had only one of their major bile ducts obstructed (10, 11). The uptake in the obstructed and the non-obstructed lobes could then be compared in a situation in which the changes in plasma bile acids and plasma lipids were much less pronounced than in the rats with total obstruction. The uptake by the obstructed and the non-obstructed parts of the liver did, however, not differ significantly, and the effect on the plasma clearance was much less than with complete obstruction. Cholestasis per se thus did not inhibit remnant uptake in the presence of a liver portion with intact bile secretion, which prevented the drastic changes in plasma composition that occur with complete obstruction. This is notable, since also incomplete cholestasis causes pronounced morphological changes in the obstructed lobe (10), for instance in the Golgi apparatus, which may be involved not only in secretory functions but also in endocytic degradative processes (4).

While the plasma cholesterol and phospholipid increase was much larger after 48 h than after 8 h of obstruction, the plasma bile acids were high but similar in both groups (Table I). The influence on the remnant clearance was similar in the two groups and was thus not proportional to the amount of abnormal lipoproteins in plasma but was rather seen in all animals with high bile acid levels in plasma. Recently, Middelhoff et al. (19) demonstrated a binding to, and an influence on, the normal structure of high-density lipoproteins of bile acids in human cholestatic plasma. The possibility that bile acids interfere with some mechanism necessary for rendering chylomicron remnants susceptible to uptake in the liver or with the cellular uptake of remnants by the liver must be considered in further studies of the mechanism

behind the inhibition of the chylomicron remnant clearance.

The defective remnant uptake, in combination with a decreased cholesterol transport by the intestinal lipoproteins, may also contribute to a defective feedback regulation of (26), and thereby to the increase in, hepatic sterol synthesis seen in cholestasis (11, 15, 32). The increased sterol synthesis is, however, also seen in the obstructed lobe in partial bile duct obstruction (11). In the same model no difference in remnant uptake was seen between obstructed and nonobstructed lobes in the present study (Table II). Other factors than a decreased flow of remnant cholesterol into the liver cell may thus contribute to the increased sterol synthesis in cholestasis.

From a clinical point of view it is worth noting that a decreased hepatic remnant uptake in cholestasis prevents the uptake and processing of fat-soluble vitamins by the liver. Hereby any deficiency state of fat-soluble vitamins in cholestasis might be aggravated.

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FACTORS AFFECTING THE IMPAIRED HEPATIC UPTAKE OF CHYLOMICRON
REMNANTS IN THE CHOLESTATIC RAT

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SUMMARY

The hepatic uptake of chylomicron remnants is severely inhibited in rats in which the bile duct has been ligated for 48 hrs. The reduced uptake is not due to the cholestasis per se, since equal amounts of chylomicron remnant lipids were recovered in different parts of the liver after ligation of only one of the two major bile duct branches (B. Larsson, Å. Nilsson, 1980, *Scand. J. Gastroent.* 15, 959-967).

In the present study a significant reduction of the hepatic chylomicron remnant uptake could be induced by intravenous infusion of bile or of large amounts of sodium taurocholate, causing far less drastic changes in plasma lipid concentrations than bile duct ligation. Bile duct ligation did not influence the clearance from plasma of ^{125}I -labelled human low density lipoproteins and induction of the apoB,E-receptor by treatment with ethinylestradiol before bile duct ligation did not prevent the marked decrease in hepatic remnant uptake.

Perfused livers from bile duct ligated rats metabolized chylomicron remnants at a reduced rate. Rat hepatocyte monolayer cultures metabolized remnants prepared by injecting chylomicrons in cholestatic rats as well or better than remnants prepared in hepatectomized animals. Cholestatic serum was more effective than normal serum in inhibiting the catabolism of chylomicron remnants in the cell cultures.

The results indicate that the reduced hepatic uptake of chylomicron remnants in the cholestatic rat is not due to formation of abnormal remnant particles. Rather, plasma changes secondary to the cholestasis, such as the accumulation of bile acids and pathological lipoproteins seem to interfere with the catabolism of chylomicron remnants.

INTRODUCTION

We have previously demonstrated that the hepatic uptake of chylomicron remnant lipids is severely reduced in the cholestatic rat (1). This is not correlated with the morphological changes in the cholestatic liver (2,3), since equal amounts of chylomicron lipids are recovered in different parts of the liver after ligating only one of the bile duct branches (1,4). Nor is it likely to depend on a defective formation of chylomicron remnants in the cholestatic rat, since the hepatic uptake is inhibited to a similar extent, regardless of whether chylomicrons or preformed chylomicron remnants are injected (1).

The reason for the reduced uptake of chylomicron remnants in the liver of bile duct ligated rats should thus be sought either in the cholestatic changes in plasma or in effects on the liver secondary to the plasma changes. For instance, pathological lipoproteins of cholestatic rat plasma might interfere with the binding of chylomicron remnants to the liver. Since the hepatic uptake of chylomicron remnants is generally assumed to occur via the apoE-receptor (5,6), it is of interest that the total plasma apoE concentration is significantly elevated in the cholestatic rat (7). For example, the pathological lipoprotein-X, secreted from the cholestatic rat liver, contains apoE as its major protein (8).

Several other pathological lipoproteins have been described in cholestasis. One of these which has been demonstrated in man and rat, is a triacylglycerol-rich LDL called β_2 -LP (9) or LP-Y (10,11). It has been suggested that this particle is derived from chylomicron remnants accumulating in cholestatic plasma (9),

although there are also indications that it is secreted directly from the cholestatic liver (8). The apolipoprotein composition of this particle has not been described.

Furthermore, the exposure of the liver to the cholestatic plasma might reduce the hepatic capacity for remnant uptake, either by a non-specific membrane effect or by a specific down-regulation of a lipoprotein receptor. Acute bile salt infusion in the dog (12) decreases the activity of the hepatic apoB,E-receptor, while the apoE-receptor has been fairly resistant to metabolic regulation (5,12).

In this study we examined some of the possible mechanisms underlying the inhibited chylomicron remnant uptake in the cholestatic rat liver. Such information would be relevant for the general understanding of lipoprotein receptor function under pathological conditions. It would also be of clinical interest in relation to the metabolism of fat-soluble vitamins in the cholestatic state. For instance, a reduced hepatic uptake not only of retinyl esters (1) but also of vitamin D has been reported in the cholestatic rat liver (13).

MATERIALS AND METHODS

Materials.

Male Sprague-Dawley rats (Anticimex AB, Stockholm, Sweden) weighing 250-300 g were used. [$1\alpha,2\alpha$ - ^3H]cholesterol was obtained from Amersham, Bucks, Great Britain and [^3H]retinol (all-trans) from New England Nuclear, Dreieich, West Germany. Ethinylestradiol was a kind gift from Leo AB, Helsingborg, Sweden.

Methods.

Radioactively labelled chyle was prepared as described earlier (1). Chylomicron remnants were prepared either in vivo by injecting chylomicrons ($S_f > 400$) into eviscerated rats (14) or in vitro by incubating chyle with post-heparin plasma (15). Chylomicron remnants were isolated by ultracentrifugation as described in (16). ^{125}I -labelled human LDL was generously provided by Dr. C.H. Florén, Lund.

Liver perfusions were performed as described earlier (17). After an initial rinse with 100 ml Krebs-Ringer buffer, livers were perfused for 1 h with 40 ml of a recycling medium, containing [^3H]cholesterol-labelled chylomicron remnants, 3% bovine serum albumin, 0.1% glucose and 22% washed human erythrocytes in Krebs-Ringer buffer. In the normal livers the bile ducts were cannulated, while the bile duct ligature was kept intact in the cholestatic livers. At the end of experiments 50 ml cold buffer was passed through the livers before homogenisation of the entire organ in saline. Aliquots of the 10% homogenate were then extracted and further analyzed. The preparation of hepatocyte monolayer cultures, as well as the method of calculating uptake and hydrolysis followed the description given by Florén and Nilsson (18). Cholestasis was performed by ligating the common bile duct 2 days before the experiments. All animals had free access to food and water.

Analytical.

Lipid extractions and radioactivity determinations were carried out as previously described (1). Triacylglycerols and plasma cholesterol was determined using enzymatic kit methods (Boehringer Mannheim GMBH, West Germany). Free and esterified cholesterol in lipoproteins was determined (19) after separating the fractions by t.l.c. (1) before saponification (20). Plasma phosphatidylcholine was determined by a phospholipase D-choline

oxidase kit method (Phospholipids B-test, Wako Chemicals, Osaka, Japan). Bile acids were determined using a fluorimetric method (Nyegaard & Co. A/S, Oslo, Norway).

RESULTS

Metabolism of cholestatic chylomicron remnants in normal hepatocytes

[^3H]cholesterol-labelled lipoproteins were collected in the $d < 1.006$ g/ml fraction of plasma from cholestatic rats that had been injected with [^3H]cholesterol-labelled chyle 30-60 min earlier. Although less than 2% of the plasma cholesterol and phospholipids was collected in this fraction in cholestatic rats not injected with chyle, 10-50% of the material isolated with the radioactively labelled remnants could be contributed by endogenous lipoproteins. As judged from the radioactivity data, the cholestatic chylomicron remnants were taken up in hepatocyte monolayer cultures and their cholesteryl ester content hydrolyzed at a rate comparable to that of normal chylomicron remnants, produced in eviscerated rats; this was even observed in experiments where the latter particles had lost a greater fraction of their initial triacylglycerol content (Fig. 1). Incubation of cholestatic remnants with normal serum, or normal remnants with cholestatic serum, prior to the incubation with hepatocytes did not influence the rate of metabolism of the different lipoproteins (Fig. 2). In keeping with earlier findings (21), the presence of serum during the incubation with hepatocytes in monolayer cultures inhibited the chylomicron remnant metabolism (Fig. 3). Cholestatic serum, having a several-fold higher cholesterol content, was somewhat more inhibitory than normal serum even when equal cholesterol concentrations were compared.

Uptake of chylomicron remnants in perfused livers from normal and cholestatic rats

The uptake of normal chylomicron remnants was not significantly reduced in livers from cholestatic rats when perfused for 1 h with a serum-free medium. However, the fraction of remnant [^3H]cholesteryl esters hydrolyzed in the cholestatic livers was 40% lower than in control experiments where normal livers were perfused (Table I). The uptake of intact chylomicron remnant [^3H]cholesteryl esters was also studied in non-recycling perfusions where the livers were perfused with 5-250 μg of lipoprotein cholesterol for only 1-3 min. In these experiments the fraction of [^3H]cholesteryl esters found associated with the cholestatic livers was $70 \pm 20\%$ ($n=6$) of that in normal livers perfused with corresponding concentrations of chylomicron remnants. This difference is not statistically significant.

Infusion of bile or bile salts

An attempt was made to change the plasma composition in a manner resembling the cholestatic state without primarily interfering with the liver function. For this purpose pooled rat bile, collected during the first 6 hrs after establishing a bile fistula, was administered intravenously to normal rats during 1 h. The dose given roughly corresponded to fourfold the normal bile production during this time. The bile infusion induced a significant increase in both plasma cholesterol and triacylglycerols (Table II), although the very high levels seen after two days of bile duct ligation (1) were not reached. The bile acid concentration in plasma was significantly increased by the infusion, although less than 0.3% of the administered dose was recovered in plasma. The rise in plasma cholesterol by 85% was, however, about five-fold higher than expected from the amount of bile infused and a similar discrepancy could be calculated for the increase in phosphatidylcholine. The hepatic uptake of [^3H]retinol-labelled chylomicron remnants was reduced by 35%

during a 10 min period immediately following the bile infusion (Table II). The effect of acute intravenous bile infusion was thus comparable to the reduction by 60% of the hepatic remnant uptake seen in cholestatic rats in vivo (1).

A less pronounced, but significant, degree of inhibition of the hepatic remnant uptake could be induced by administering similar amounts of bile acids as infused with the bile in the form of pure sodium taurocholate solution (Table II). The reduction of hepatic uptake in this case occurred in the absence of an increase in plasma lipid concentrations. Yet, the infusion of increasing amounts of sodium taurocholate correlated well with the reduction of the hepatic remnant uptake (Table II).

LDL metabolism

The clearance from plasma of ^{125}I -labelled human LDL was not impaired in the cholestatic rats (Fig. 4). Induction of the hepatic apoB,E-receptor with ethinylestradiol (22-24), did not abolish the inhibiting effect of bile duct ligation on hepatic chylomicron remnant uptake (Table III). Also estrogen treatment did not affect the hepatic uptake of chylomicron remnants in rats not subjected to bile duct ligation (Table III).

DISCUSSION

The chylomicron remnants formed in the cholestatic rat were metabolized as normal remnants both when injected in vivo (1) and when incubated with hepatocyte cultures (Fig. 1). In fact it is suggested from Figs. 1 and 2 that the cholestatic remnants are taken up in hepatocytes more avidly than normal chylomicron remnants of similar size. Preincubation of remnants with plasma from cholestatic rats did not influence their subsequent uptake in the hepatocyte monolayers (Fig. 2). In line with the earlier in vivo experiments (1), showing that also the clearance of

normal preformed remnants is strongly inhibited in cholestasis, the hepatocyte experiments thus speak against the formation of defective remnant particles as a cause of the slow remnant clearance in cholestasis. Although the total plasma concentration of apoE is increased in the cholestatic rat (7) the increase is largely confined to the denser lipoprotein fractions, the apoE content in VLDL being extremely low (25). Other investigations suggest a decreased apoC content in cholestatic VLDL (26). An increased apoE/apoC ratio in the cholestatic remnants would, if present, be expected to promote the uptake of these lipoproteins by the hepatocytes (27). Although the exact composition of the chylomicron remnants formed in the cholestatic animal has not been determined, our results indicate that the cholestatic rat might actually be used as a simple and suitable model for producing large amounts of chylomicron remnants.

The greater degree of inhibition exerted by cholestatic than by normal serum on the metabolism of chylomicron remnants in hepatocytes (Fig. 3), could at least partly be attributed to the presence of accumulating remnant particles in the cholestatic serum. The rise in plasma cholesterol seen after ligating the bile duct (1) exceeded, however, the amount of cholesterol transported with chyle during the same period in rats with a bile fistula (B. Landin, Å. Nilsson, unpublished data). Similar mechanisms are thus probably working in the cholestatic rat and in the rat receiving bile intravenously, in both cases rapidly leading to a mobilisation of cholesterol and phospholipids to the plasma. This phenomenon has also been described in dogs having their bile duct inserted into the vena cava (28). If much of the lipid excess is present in pathological lipoproteins also containing apoE, it might contribute to the capacity of cholestatic serum to inhibit the chylomicron remnant binding to the liver cells.

After infusion of taurocholate instead of native bile the hepatic remnant uptake was reduced, although no significant increase in plasma cholesterol or phospholipids occurred (Table II). The bile salt load in itself might thus cause a suppression of the hepatic remnant uptake, on which the effect of competing lipoproteins might be superimposed in cholestatic or bile infused animals. The mechanism by which the effect of the bile salts is mediated is not clear. Increased bile acid levels in plasma does not invariably correlate with a suppression of chylomicron remnant uptake. For instance, in porta-caval shunted rats, which have a significantly increased concentration of plasma bile acids, the hepatic uptake of remnants is actually increased (16). Also infusion of taurocholate into dogs did not suppress the apoE-receptor expression in the liver, whereas the apoB,E-receptor activity was markedly reduced (12). The expression of this latter receptor is, however, low in the normal rat (22) and it is unlikely to have any major role in chylomicron remnant degradation (Table III).

In the present experiments the elimination of ^{125}I -labelled human LDL was not retarded by bile duct ligation (Fig. 4). In both normal and cholestatic rats the half-life of injected LDL between 4 and 24 hrs after injection was 8 hrs, thus corresponding to the results reported by Stein et al. (29) using LDL labelled in the cholesteryl ester moiety. Although clearance of heterologous LDL from plasma may not necessarily reflect variations in the hepatic lipoprotein uptake (30), suppression of the apoB,E-receptor is an extremely unlikely cause of the inhibited remnant clearance in cholestasis.

The degradation of chylomicron remnant cholesteryl esters was decreased in perfused cholestatic livers, whereas the amount of chylomicron remnants found associated with the liver was not reduced (Table I). This may be interpreted in different ways. The

apoE-receptor mediated binding leading to catabolism of the remnants may actually be suppressed, whereas non-specific binding to liver cell surfaces is less affected. The possibility that bile duct ligation causes a suppression of apoE-receptor function thus remains. It should, however, also be considered that the cholestatic state might inhibit the hepatic remnant uptake by interfering with the internalization process rather than by influencing the lipoprotein receptor expression. In this case also the clearance of other compounds that are normally metabolized by receptor-mediated endocytosis in the hepatocytes would be impaired. In fact the concentration of asialoglycoproteins is elevated in plasma from patients with cholestatic jaundice (31). It would thus be of interest to further study the uptake of e.g. asialoglycoproteins in perfused cholestatic livers. Also, it would be important to determine the contribution by different cell types in the liver to the uptake, since in the cholestatic livers the uptake of chylomicron remnants does not exceed what could be accounted for by uptake solely in the non-parenchymal liver cells.

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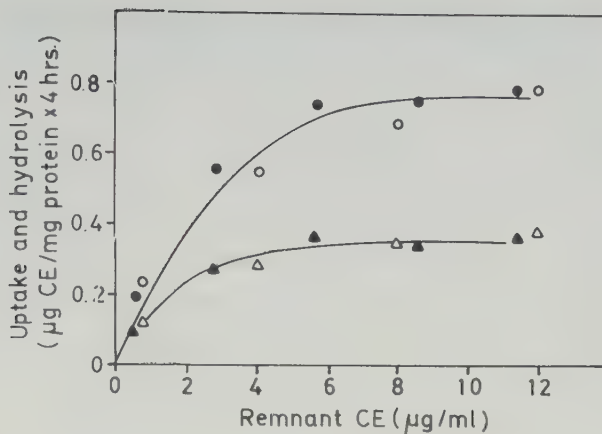


Fig. 1 Uptake and degradation of normal and cholestatic chylomicron remnants in rat hepatocytes

Increasing doses of [^3H]cholesteryl ester (CE) labelled chylomicron remnants obtained from cholestatic rats (●▲) or eviscerated normal rats (O Δ) were incubated with hepatocyte monolayers (4.0 mg protein per dish) in a total volume of 2.5 ml for 4 hrs. The cholestatic remnants contained per ml 146 µg CE (32×10^3 cpm ^3H) and 202 µg unesterified cholesterol (UC) (8.4×10^3 cpm ^3H). Corresponding values for the normal remnants were 187 µg CE (64×10^3 cpm ^3H) and 125 µg UC (19.9×10^3 cpm ^3H). Both types of remnants were prepared from the same batch of chyle. The proportion of initial triacylglycerols hydrolyzed was 66% in the cholestatic and 84% in the normal remnants. The total uptake (O●), i.e. the sum of [^3H]CE cell-associated and hydrolyzed, and the hydrolysis (Δ▲) was calculated as in (18). Values indicated are means from duplicate incubations.

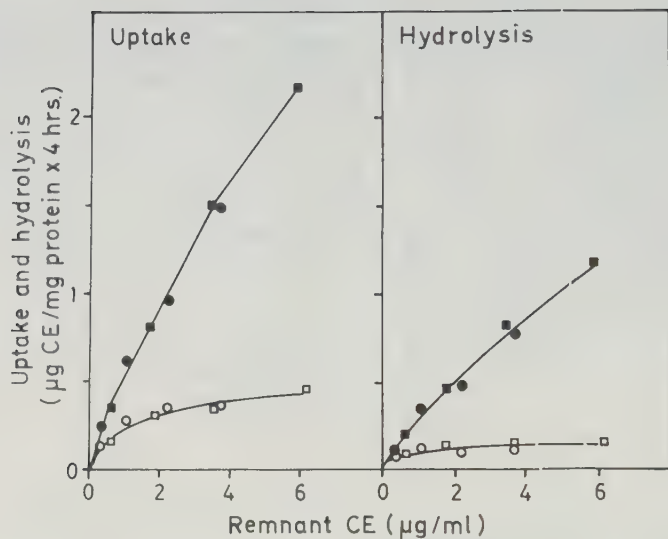


Fig. 2 Effect of incubation of chylomicron remnants with serum on remnant metabolism in hepatocyte cultures

$[^3\text{H}]$ cholesterol-labelled chylomicron remnants were prepared in cholestatic rats or by in vitro incubation with post-heparin plasma. Aliquots of cholestatic remnants (96% triacylglycerols hydrolyzed) or normal remnants (84% hydrolyzed) were incubated at 37° for 1h with normal or cholestatic rat serum, respectively. The remnants contained 250 μg cholesterol in a volume of 1.35 ml which was mixed with 4.05 ml serum. The serum-incubated remnants were then reisolated by ultracentrifugation before incubation with hepatocytes (2.3 mg protein per dish) for 4 hours in a total volume of 2.5 ml serum-free medium. The metabolism of cholestatic and normal remnants that had not been subjected to incubation with serum as described above were studied in parallel hepatocyte incubations.

The different types of chylomicron remnants added to the hepatocytes contained per ml: normal remnants ($\circ$), 38 μg cholesteryl esters (CE) (17×10^3 cpm ^3H) and 25 μg unesterified cholesterol (UC) (2.8×10^3 cpm ^3H); normal remnants incubated with cholestatic serum ($\square$), 62 μg CE (25×10^3 cpm ^3H) and 63 μg UC (2.4×10^3 cpm ^3H); cholestatic remnants ($\bullet$), 38 μg CE (17.4×10^3 cpm ^3H) and 53 μg UC (2.2×10^3 cpm ^3H); cholestatic remnants incubated with normal serum ($\blacksquare$), 59 μg CE (27×10^3 cpm ^3H) and 117 μg UC (2.4×10^3 cpm ^3H). The values for total $[^3\text{H}]$ CE uptake (left panel) and $[^3\text{H}]$ CE hydrolysis (right panel) are based on duplicate incubations.

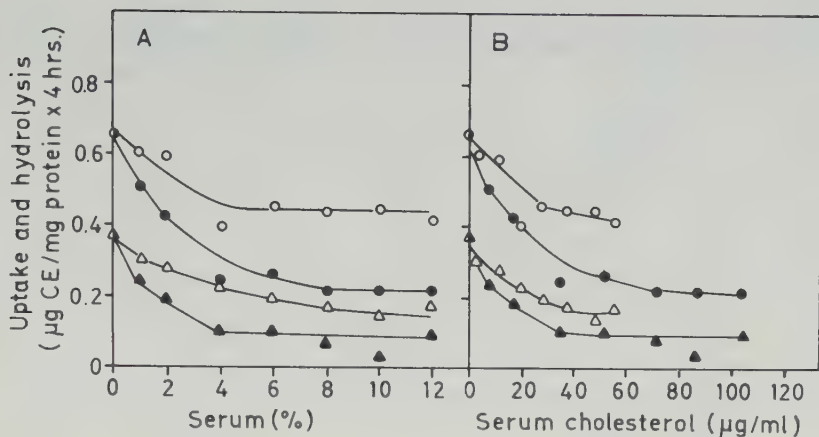


Fig. 3 Effects of normal and cholestatic serum on uptake and degradation of chylomicron remnants in hepatocytes

Fresh serum from normal or cholestatic rats was heated to 56° for 30 min and then dialyzed against saline at +4° over night. The normal serum (○ △) contained 306 µg cholesteryl esters (CE) per ml and 168 µg unesterified cholesterol (UC) per ml. The cholestatic serum (● ▲) contained 420 µg CE per ml and 450 µg UC per ml. Increasing amounts of serum were added to hepatocyte cultures (3.6 mg protein per dish) when incubated for 4 hrs in a volume of 2.5 ml with [³H]CE-labelled chylomicron remnants prepared in eviscerated rats (85% of initial triacylglycerols hydrolyzed) containing 6.8 µg CE (8.9x10³ cpm ³H) and 7.0 µg UC (2.1x10³ cpm ³H). The values (means from duplicate incubations) of total uptake (○ ●) and hydrolysis (△ ▲) of remnant [³H] CE vs. serum volume added given in panel A, are replotted vs. total serum concentration (CE+UC) in the incubations in panel B.

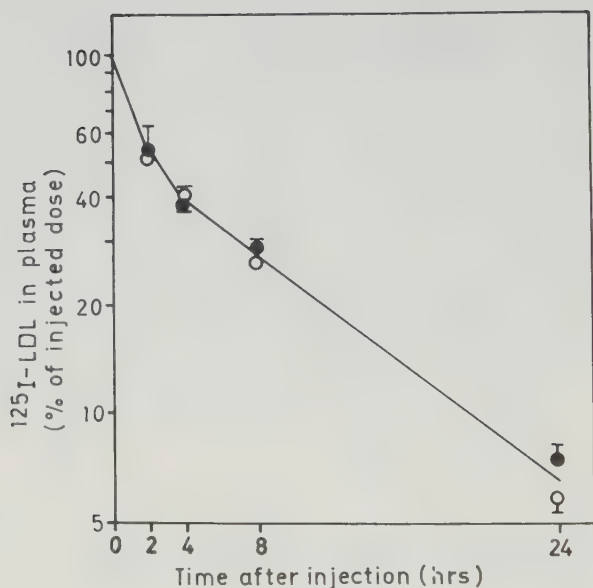


Fig. 4 Elimination of human ^{125}I -LDL from plasma of normal and cholestatic rats

65 μg of LDL protein containing 71 000 cpm ^{125}I was injected intravenously. Aliquots of plasma were precipitated with TCA before the radioactivity determinations. Open symbols (O) represent normal rats; filled symbols (●) cholestatic animals. Values are means $\pm$ S.E.M. for three rats of each type at each time point studied.

Table I

Uptake and degradation of [^3H]cholesterol labelled chylomicron remnants in perfused livers from normal or cholestatic rats

Perfusate cholesterol ($\mu\text{g/ml}$)	Total [^3H]CE uptake			[^3H]CE hydrolyzed		
	(% of added)	(% of control)	(% of control)	(% of added)	(% of control)	(% of control)
control	cholestasis	cholestasis	control	cholestasis	cholestasis	cholestasis
8	59	25	42	22	9	39
14	17	16	94	5	4	78
19	29	31	104	14	10	67
40	44	25	58	19	11	57

Livers were perfused for 1 h with a recycling medium (see Methods section) containing [^3H]cholesterol-labelled chylomicron remnants prepared in eviscerated rats. During the preparation 67-88% of the initial lymph triacylglycerol content was hydrolyzed as compared to the cholesteryl ester content. At the end of experiments $98 \pm 1\%$ ($n=4$) of the added ^3H radioactivity was recovered in liver and perfusate together. When livers perfused with chylomicron remnants from the same preparation were compared, on average $86 \pm 22\%$ of the amount of [^3H]cholesteryl esters (CE) associated with the control livers was found in the cholestatic livers. Expressed in a similar manner $60 \pm 8\%$ of the [^3H]CE hydrolyzed in normal livers was hydrolyzed in the cholestatic livers ($p<0.05$). Total uptake was calculated as the sum of CE cell-associated and hydrolyzed. Values are from 1-2 rats at each concentration.

Table II

Effects of intravenous infusion of bile or bile acids on plasma lipid concentrations and on hepatic uptake of chylomicron remnants.

Expt	Bile acids infused ($\mu\text{mol}/100 \text{ g rat}$)	Plasma lipids			Bile acids (nmol/ml)	Hepatic recovery of [^3H]retinol (%)
		Cholesterol ($\mu\text{g}/\text{ml}$)	Triacyl- glycerols ($\mu\text{g}/\text{ml}$)	Phosphatidyl- choline ($\mu\text{g}/\text{ml}$)		
A. Saline	0	409 $\pm$ 44	1031 $\pm$ 45	1002 $\pm$ 110	16 $\pm$ 3	85 $\pm$ 4
Bile	63	762 $\pm$ 45**	1556 $\pm$ 108*	1267 $\pm$ 93	50 $\pm$ 10*	46 $\pm$ 2**
B. Saline	0	503 $\pm$ 32	1090 $\pm$ 132	1158 $\pm$ 123	13 $\pm$ 5	99 $\pm$ 4
NaTC	70	504 $\pm$ 31	942 $\pm$ 127	1181 $\pm$ 70	49 $\pm$ 13	86 $\pm$ 2*
C. Saline	0	608	471	1454	16	76
NaTC	22	586	388	1243	19	73
"	47	634	308	1264	11	54
"	87	629	329	1570	49	55

Rats weighing 130-275 g were injected intravenously with native rat bile (Expt. A) or sodium taurocholate (NaTC) in phosphate-buffered saline (Expt B and C). An initial bolus of 1 ml (Expt C) or 2 ml (Expt A and B) was followed by an infusion of 2.4 ml during 60 min. Control rats received corresponding volumes of buffer. At the end of the infusion 200 μl [^3H]retinol-labelled chyle (Expt C) or 750 μl in vitro prepared chylomicron remnants (Expt A and B) was injected. Plasma lipids and the recovery of [^3H]retinol (free and esterified) in the liver was determined 10 min (Expt A and B) or 30 min (Expt C) after the lipoprotein injection. The lipoprotein composition was: in expt A, 151 μg cholesterol, 655 μg triacylglycerols (17% of initial), 42225 cpm [^3H]retinyl esters and 1613 cpm [^3H]retinol; in expt B 313 μg cholesterol, 205 μg triacylglycerols (12% of initial), 19238 cpm [^3H]retinyl esters and 825 cpm [^3H]retinol; in Expt C 68 μg cholesterol, 1.10 mg triacylglycerols, 1587 cpm [^3H]retinyl esters and 538 cpm [^3H]retinol. The bile infused contained 164 μg cholesterol, 3.4 mg phosphatidylcholine and 38 μmol bile acids per ml. The values are means from two rats or means $\pm$ SEM from 3 rats. Data were compared using Student's t-test. * $p < 0.05$, ** $p < 0.01$.

Table III

Effects of ethinylestradiol-treatment and bile duct ligation on plasma lipid concentrations and chylomicron [^3H]cholesteryl ester uptake in the liver

Ethinylestradiol	Bile duct ligation	Cholesterol ($\mu\text{g/ml}$)	Triacylglycerols ($\mu\text{g/ml}$)	Hepatic recovery of [^3H]CE
-	-	221 $\pm$ 21	798 $\pm$ 174	55 $\pm$ 2.8
+	-	91 $\pm$ 15	241 $\pm$ 16	51 $\pm$ 2.9
-	+	2090 $\pm$ 131	1306 $\pm$ 137	19 $\pm$ 0.9
+	+	1167 $\pm$ 64	187 $\pm$ 31	19 $\pm$ 2.3

P estradiol-treated vs non-treated

ligated	< 0.001	< 0.001	n.s.
non-ligated	< 0.010	< 0.050	n.s.

P ligated vs non-ligated

treated	< 0.001	n.s.	< 0.010
non-treated	< 0.001	n.s.	< 0.001

Groups of rats were injected daily with 5 mg/kg 17 α -ethinylestradiol in propyleneglycol or pure propyleneglycol. After 7 days of treatment the rats in two of the groups were subjected to bile duct ligation. Two days later the animals were injected with 1 ml [^3H]cholesterol-labelled chylomicrons containing 86 μg cholesteryl esters (179×10^3 cpm ^3H), 32 μg cholesterol (66×10^3 cpm ^3H) and 5.2 mg triacylglycerols. The hepatic recovery of [^3H]cholesteryl esters was determined 20 min later. Values are means $\pm$ S.E.M. from 3 rats (radioactivity data) or 5 rats (lipid data). Data were compared using Student's t-test. n.s. = not significant.

